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THIAMINE, ITS INTERMEDIATES AND GROWTH OF *CORTICIUM MICROSCLEROTIA* (MATZ) WEBER

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Received February 25, 1953

(Communicated by Prof. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

Numerous investigators have shown that many fungi are unable to grow normally on strictly synthetic media, unless minute amounts of one or more growth substances are supplied. The considerable amount of literature that has accumulated on the science of growth substances in relation to fungi has been reviewed by Robbins and Kavanagh (1942), Schopfer (1943), Fries (1948), Hawker (1950) and Lilly and Barnett (1951). Thiamine deficiency is the most common among filamentous fungi, but not all thiamine heterotrophic fungi require the entire thiamine molecule. Fungi like *Phytophthora* spp. require an external supply of intact thiamine for their growth (Robbins, 1938) while *Phycomyces blakesleeanus* is able to grow if supplemented with both the intermediate fractions of thiamine, namely, pyrimidine and thiazole (Schopfer and Jung, 1937; Robbins and Kavanagh, 1937; Sinclair, 1937). The same is true of some *Torula* spp. (Robbins and Kavanagh, 1938 *a*). Other fungi like *Rhodotorula rubra*, *Parasitella simplex*, *Dematium nigrum* (Schopfer, 1937, 1938), *Torula* spp., *Pythium butleri*, *Sclerotium rolfsii* (Robbins and Kavanagh, 1938 *a*, *b*, *c*), and *Tricholoma* spp. (Norkrans, 1950) require only pyrimidine, while growth of *Mucor ramannianus* (Mueller and Schopfer, 1937) is promoted by thiazole alone. That the synthesis of thiamine is accomplished by these fungi from any one or both the intermediate fractions supplied is a definitely established fact (Robbins and Kavanagh, 1938 *a*; Bonner and Buchman, 1939; Leonian and Lilly, 1940). The results of similar investigations with the fungus *Corticium microsclerotia* (Matz) Weber, are reported here.

Materials and Methods

All general precautions necessary in studies with growth substances were taken.

Source and maintenance of cultures.—The following pure cultures of fungi were obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland, and the names followed are as given in their collection: *Corticium*

koleroga (Cooke) v. Höhnel, *Corticium stevensii* Burt, *Corticium microsclerotia* (Matz) Weber, *Hypochnus solani* Prill. and Del. and *Hypochnus sasakii* Shirai. A culture of *Pellicularia filamentosa* (Pat.) Rogers [= *Corticium solani* (Prill. and Del.) Bourd. and Galz.] isolated by Ramakrishnan, T. S. and Ramakrishnan, K. (1948) from *Maranta arundinacea* was obtained from the latter author. A culture of *Pellicularia koleroga* Cooke [= *Corticium koleroga* (Cooke) v. Höhnel] (Rogers, 1943) was isolated by the author from leaves of *Coffea arabica* L. (Mathew, 1952). All cultures were maintained on 2% potato sucrose agar slants.

Glassware.—Pyrex vessels were used throughout and these were thoroughly treated with sulphuric acid-potassium dichromate cleaning solution, washed with several changes of tap water, finally rinsed with distilled water and dried.

Water.—Distilled water was re-distilled in an all-pyrex still and this was used in all experiments.

Chemicals.—All chemicals used were of the highest purity: KNO_3, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, B.D.H. 'Analar'; glucose, Baker's analysed; thiamine hydrochloride, riboflavine, pyridoxine hydrochloride, pantothenic acid (calcium salt), nicotinic acid, B.D.H.; (+) biotin, pyrimidine (2-methyl-4-amino-5-aminomethyl-pyrimidine-dihydrochloride), thiazole (4-methyl-5-hydroxyethyl-thiazole), Hoffmann La Roche; inositol, Polychemical Co. and *p*-aminobenzoic acid, International Chemical Corporation.

Purification of agar.—Agar was purified by treating it with 5% aqueous pyridine (Robbins, 1939).

Preparation of media.—The basal medium I *a* had the following composition: KNO_3 2 g., KH_2PO_4 1 g., $MgSO_4.7H_2O$ 0·5 g., glucose 20 g. and water to make 1,000 ml. The same medium solidified with 2% purified agar is referred as medium I *b*. The growth substances were added to the medium before autoclaving and sterilised at 10 pounds pressure for 15 minutes. The pH of the media after the different treatments varied between 5 to 6 at the time of inoculation. No trace elements were added since tests with *Aspergillus niger* showed that these media prepared with the care mentioned were not essentially free of the trace elements Fe, Cu, Mn, Zn, Mo and Ga (Steinberg, 1919, 1935, 1936, 1937, 1938) necessary for its normal growth and sporulation.

Inoculation.—Two or three minute sclerotia were removed from potato sucrose agar slants and used for inoculation, care being taken not to introduce any agar.

Incubation.—All cultures were incubated at the room temperature which varied between 26–30° C. during the experimental period.

Mycelial dry weight determinations.—Conical flasks of 250 ml. capacity with 20 ml. medium I *a*, were used as containers. During the early stages of growth the cultures were visually compared at intervals and arbitrary ratings given and this method was satisfactory in detecting any large differences in the rate of growth of the fungus. When growth of the fungus was near maximum the dry weights were determined. The mycelial mats were harvested into filter-papers, washed thoroughly with distilled water, dried at 55–60° C. for 72 hours and cooled in a desiccator. The dry mycelial mats were easily transferred to a previously weighed dry crucible and weighed on an analytical balance. The mean values of four replicates each with their standard error, are presented.

Experimental

Growth substance requirements of some fungi.—The fungus *Pellicularia koleroga* Cooke [= *Corticium koleroga* (Cooke) v. Höhnel] is thiamine heterotrophic (Mathew, 1952). According to Rogers (1943), *Corticium koleroga* and *C. stevensii* Burt are synonyms of *Pellicularia koleroga* Cooke while C. *microsclerotia* (Matz) Weber, *C. solani* (Prill. and Del.) Bourd. and Galz. and *Hypochnus solani* Prill. and Del. are synonyms of *Pellicularia filamentosa* (Pat.) Rogers. Others have considered *H. sasakii* Shirai to be identical with *C. solani* (Hemmi and Endo, 1933; Ryker, 1938).

Whether some of these closely related fungi, have similar growth substance requirements was tested. They were grown in two series of test-tubes, one containing medium I *b* and the other the same medium with malt extract added at the rate of 1 g. per 1,000 ml.

C. stevensii, H. solani, H. sasakii and the isolate of *P. filamentosa* (Ramakrishnan *et al.*, 1948) grew equally well in both the series while *C. koleroga* (Baarn isolate), the isolate of *P. koleroga* (= *C. koleroga*) the author's isolate (Mathew, 1952) and *C. microsclerotia* made only negligible growth in media without malt. It was deduced that the last mentioned three fungi are heterotrophic for one or more growth substances present in malt extract and in this article results of further experiments on the growth substance requirements of *Corticium microsclerotia* are presented. The results also showed that some of the fungi, considered by Rogers (1943) to belong to a single species, are physiologically different.

Effect of exclusion of a single growth substance from a mixture 'M' on the growth of Corticium microsclerotia, *in medium I b.*—The mixture 'M'

had the following composition: thiamine, riboflavine, pyridoxine, pantothenic acid, nicotinic acid, *p*-aminobenzoic acid each 2 μg., biotin 0·1 μg., inositol 2 mg. and this was added to 5 ml. of medium in each test-tube. A single growth substance was excluded from the mixture 'M' in each of the other treatments. In addition to the controls to which no growth substance was added, another series of tubes with malt extract added at the rate of 1 g. per litre, was also maintained for comparison.

In the controls the growth of the fungus was negligible. When thiamine was withdrawn from the mixture 'M' the growth of the fungus although slightly better than the controls, was still negligible. The exclusion of any of the other growth substances from the mixture did not affect the growth of the fungus and in all those tubes numerous, minute, hard, black or dark brown sclerotia were formed in about 5 days, covering the entire slant. In the presence of malt extract growth although good, was slightly inferior to the tubes containing the growth substance mixture. It was concluded that the fungus has a total deficiency for thiamine.

Period of incubation to reach maximum growth.—To 20 ml. of medium I *a* in each flask, thiamine was added at the rate of 2 μg. per flask. The dry weight of the fungus was determined at 5-day intervals.

The growth of the fungus was slow for the first 5 days and at the end of this period only a few scattered sclerotia were seen. Mycelial growth and sclerotial formation was rapid from the 5th to the 15th day and this resulted in considerable increase in the dry weight of the fungus, the maximum being recorded on the 15th day (Table I). Autolysis and a consequent reduction in the weight was noticed on the 20th day, but further loss in the weight of the fungus recorded on the 25th and 30th days was very little. The weights remained nearly constant because the bulk of the dry weight of this fungus is the weight of its sclerotia and autolysis does not affect these, as it does the mycelium.

Optimum amount of thiamine for growth.—To medium I *a*, the following amounts of thiamine were added per flask: none, 0·001 μg., 0·01 μg., 0·1 μg., 1·0 μg., 2·0 μg. and 10·0 μg. The fungal mats were harvested after 15 days growth.

The growth of the fungus was negligible in the controls as well as in the presence of 0·001 μg., and 0·01 μg. per flask. The fungal growth steadily increased from 0·1 μg. and the highest weight was recorded at 2·0 μg. level (Table II). At 10·0 μg. per flask the growth of the fungus was considerably inhibited and was only equal to the growth obtained at 0·1 μg.

TABLE I

Period of incubation in days required to reach maximum growth of Corticium microsclerotia, *with the addition of* 2 *μg. thiamine to* 20 *ml. of medium I* a

Period of incubation in days	Dry weight of fungus in mg.
5	45·5 ± 7·1
10	132·5 ± 5·5
15	181·6 ± 6·4
20	148·0 ± 2·5
25	143·9 ± 3·7
30	140·9 ± 2·1
Control without thiamine (at the end of 30 days)	1·2 ± 0·2

TABLE II

Effect of addition of different amounts of thiamine to 20 *ml. medium I* a, *on growth of* Corticium microsclerotia

Amount of thiamine in each flask	Growth ratings		Dry weight of fungus in mg.
	5 days	10 days	15 days
none ..	*n*	*n*	0·9 ± 0·2
0·001 μg. ..	*n*	*n*	1·1 ± 0·3
0·01 μg. ..	*n*	*n*	3·0 ± 1·2
0·1 μg. ..	*	†	24·1 ± 2·4
1·0 μg. ..	*	†	31·9 ± 3·0
2·0 μg. ..	†	‡	125·9 ± 14·6
10·0 μg. ..	*	†	22·2 ± 2·7

n — negligibie ; * poor ; † fair ; ‡ good.

Thiamine, its intermediates and growth.—Thiazole and pyrimidine, the two intermediates of thiamine, were added in equimolar proportions. The following were the treatments: none, thiazole 0·850 μg., pyrimidine 1·252 μg., thiazole 0·850 μ g. *plus* pyrimidine 1·252 μg. and thiamine 2 μg. The dry weights were determined after 15 days growth.

The growth of the fungus was negligible in the controls and in the presence of thiazole alone, while pyrimidine alone promoted a fair amount of growth. When thiazole and pyrimidine were supplied together the growth obtained was only slightly less than that supported by intact thiamine (Table III).

TABLE III

Effect of addition of thiamine and its intermediates to 20 *ml. medium I* a, *on growth of* Corticium microsclerotia

Growth substance	Growth ratings		Dry weight of fungus in mg.
	5 days	10 days	15 days
§None ..	*n*	*n*	0·9 ± 0·2
Thiazole 0·850 μg. ..	*n*	*n*	1·5 ± 0·5
Pyrimidine 1·252 μg. ..	*	†	26·4 ± 2·5
Thiazole + Pyrimidine ..	†	‡	102·1 ± 8·2
§Thiamine 2 μg. ..	†	‡	125·9 ± 14·6

n — negligible ; * poor ; † fair ; ‡ good.

§ The last two experiments were conducted simultaneously and the readings for none and thiamine 2 μg. were taken from the same set of flasks (Tables II, III).

DISCUSSION

Corticium microsclerotia when cultured on a glucose nitrate medium failed to grow unless a trace of thiamine was added. Both in the control media to which no growth substance was added and in the series to which a vitamin mixture 'M' from which thiamine was omitted was added, the fungus made only negligible growth, while excellent growth was seen in all treatments where thiamine was present. It was also established from the same experiment that the fungus has no partial deficiency for any of the growth substances tried other than thiamine, since neither the rate nor amount of growth was reduced at the exclusion of any one of them from the mixture 'M'.

In the presence of an optimum amount of thiamine the fungus grew rapidly producing numerous sclerotia covering the entire surface, and as mentioned earlier, in this fungus the bulk of its dry weight is that if its sclerotia. This may account for the considerable difference in the mycelial weights obtained at 1·0 μg. and 2·0 μg. levels and also the rather high standard error observed at 2·0 μg. level (Table II). By the 15th day growth and sclerotial production had reached almost maximum at 2·0 μg. level, while at 1·0 μg. per flask rate of growth being slow, sclerotial formation had only just begun. Higher values could be expected at 0·1, 1·0 and 10·0 μg. levels if the flasks were to be incubated for a few more days. However, it was apparent that the optimum amount of thiamine necessary for rapid and best growth was 2·0 μg. per 20 ml. of medium. Similarly, the high standard error at 2·0 μg. level was due to the slower growth in one of the replicates where sclerotial formation did not reach a maximum, resulting in low mat weight. It was also noticed that at 2·0 μg. level the dry weight of the fungus recorded after 15 days growth (Table I) was significantly higher than that obtained in a later trial (Table II). This seems to be due to the uncontrolled fluctuations at room temperature during the two separate experimental periods, there being more warmer hours during the latter period.

Like many other thiamine heterotrophic fungi (Schopfer, 1937, 1938; Robbins and Kavanagh, 1938 *a*, *b*, *c*; Norkrans, 1950) the capacity to synthesise this vitamin is not entirely lost in the case of *C. microsclerotia* and it is able to complete the synthesis of thiamine either from a mixture of thiazole and pyrimidine or from pyrmidine alone. However, growth with pyrimidine alone is very slow and not comparable to that obtained with the pyrimidine-thiazole mixture or with thiamine. In the presence of thiazole alone, the synthesis of pyrimidine was not possible under the experimental conditions tried and the growth of the fungus was negligible as in the controls. The fungus did not sporulate in any of the media investigated.

Summary

A pure culture of the fungus *Corticium microsclerotia* (Matz) Weber, obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland, was found to be thiamine heterotrophic, when cultured on a glucose nitrate medium.

Under the cultural conditions employed the fungus was observed to reach its maximum growth in about 15 days, in the presence of an optimum amount of 2·0 μg. of thiamine per flask, containing 20 ml. of medium.

The fungus did not require the intact thiamine molecule as such and grew almost equally well when supplied with an equimolar mixture of thiazole and pyrimidine. The fungus also had the ability to slowly complete the synthesis of thiamine from pyrimidine alone, but not from thiazole.

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NOTES ON THE BIONOMICS, BREEDING AND GROWTH OF THE MURREL, *OPHICEPHALUS STRIATUS* BLOCH*

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Introduction

Considerable information is now available on the bionomics and development of the murrel, *Ophicephalus striatus* (Willey, 1910; Raj, 1916; Rahimullah, 1946; Bhattacharya, 1946; Mookerjee *et al.*, 1948). Though a fairly complete account of the life-history is available there are still several points with regard to breeding, brood care, growth, age and maturity of the species that require elucidation.

Murrel forms the bulk and mainstay of the pond fisheries in several parts of peninsular India. Though a predator, its occurrence in different types of habitats, its tenacious life, and its agreeable flesh of high nutritive value make it an important food fish which, at present, is hardly cultured on scientific lines anywhere in India. Accurate data on its growth and natural food in different stages, age, and size at maturity, etc., are of great importance in the successful cultivation of the species. Certain observations made and experiments carried out since 1945 to elucidate some of the above aspects are briefly reported in this paper.

Breeding

Observations of Thomas (1870), Day (1889), Willey (*op. cit.*), Raj (*op. cit.*), Khan (1925), Rahimullah (*op. cit.*), Jones (1946) and Mookerjee, *et al.* (1948) indicate that (*a*) intensive breeding of *O. striatus* takes place during the rainy months, (*b*) this breeding depends on the prevailing climatic conditions, the single peak period in north India including the Punjab extending from April to August, (*c*) the double peak in peninsular and southern India, corresponding to the two monsoon seasons, June-July

* Published with the kind permission of the Chief Research Officer, Central Inland Fisheries Research Station, Barrackpore, and the Director of Fisheries, Madras. Paper read before the 35th Indian Science Congress, Patna, 1948, and subsequently revised incorporating additional observations.

and November-January, and (*d*) the breeding takes place on a limited scale during other months also. From the foregoing it would appear that the species breeds all through the year. In the present study active breeding was observed from November to January in Madras; and a three-day old brood of fry was located in a pond at Azhicode, Cochin, in the middle of March. The pond at Azhicode was dewatered and desilted on 2–3–1951, and nine murrels (*O. striatus*), 6″ to 9″ long, rescued from the silt of an adjoining dry canal were released in the pond on 6–3–1951. The water in the pond was perfectly clear, 2½ feet deep and devoid of all macrovegetation. There was no rain in the locality during the period. On 14–3–1951 a brood of early fry was found in the pond, being guarded by the parent fishes. Eggs would, therefore, have been laid on 10/11–3–1951, *i.e.*, only 4–5 days after introducing the fish in the pond. The fish had spawned in spite of the fact that weeds used for building nests were not present.

The breeding habits and brood care of the murrels have also been described by several workers. According to Raj (*op. cit.*) both the parents (the male in particular) keep guard of the nest. Bhattacharya (*op. cit.*), however, states that the adult (the female more often) nurses the offspring and protects them. Disparity in size between the parents guarding the spawn or brood is very often seen. The larger, female parent is more assiduous in brood care and is constantly found with the young ones. The smaller, male parent is also seen in the vicinity, looking for intruders and chasing them away, but it quickly swims away from the brood on the slightest apprehension of danger like a passing shadow or some little disturbance. Even under circumstances of apparent danger the female stays on with the brood and will swim away only when the danger becomes imminent. It is also the first to return to the young ones after the danger is past. In the Azhicode pond the parents guarding the brood were of almost equal size.

Eggs collected from a single nest were found to be in two distinct stages of development, some of them (about 25%) with the embryo differentiated and the rest only in advanced stages of cleavage. This probably indicates that spawning takes place at intervals, as stated by Bhattacharya (*op. cit.*).

Size and Age at Maturity.—The smallest mature female specimen taken was 234 mm. long with the ovaries in the IV stage. In the Chetput Farm ponds, specimens 233·7 mm. to 317·5 mm. long which were only 9–10 months old after hatching from the egg were found to have the gonads in the IV stage. It is, therefore, clear that under natural conditions, *O. striatus* attains sexual maturity towards the end of the first year of its life. According

to Raj (*op. cit.*), Bhattacharya (*op. cit.*) and Mookerjee *et al.* (*op. cit.*) however, this murrel attains sexual maturity when two years old.

Fecundity.—According to Raj (*op. cit.*) the number of eggs varies from a few hundreds to a few thousands depending on the size of the fish; while young fry in each batch number 2,000 to 2,500 (Rahimullah, *op. cit.*). No definite account of the fecundity of the species thus appears to be available. Details of four female specimens collected from Azhicode, Cochin, and one from Serampore, West Bengal, are given in Table I.

TABLE I

Length-Weight-Fecundity data of O. striatus

No.	Date of collection	Total length (mm.)	Weight (gms.)	Stage of Maturity	Weight of Ovaries (gms.)	Volume of Ovaries (c.c.)	Total No. of Ova.	Average Diameter of Ovum (mm.)
1	26-2-1951 ..	248·0	133·0	V	6·6	6·7	4,422	1·17
2	..	247·0	135·0	IV	2·7	2·7	2,997	1·01
3	..	234·0	113·0	IV	3·4	3·3	3,883	1·01
4	22-2-1951 ..	386·0	534·0	IV	22·6	22·0	11,811	1·11
5	27-4-1949 ..	448·0	794·5	IV	30·3	30·0	20,070	..

Compared to carps the fecundity of murrels appears to be low. With increase in the size of murrels, however, the fecundity also increases. The mature egg does not vary in size in proportion to the size of the fish.

NOTES ON DEVELOPMENT

The average diameter of the laid eggs collected from the Chetput swamp, Madras, was 1·53 mm. In the early stages of embryonic differentiation the eggs are pale yellow or amber coloured, but with the formation of chromatophores on the advanced embryos they appear, to the naked eye, brownish in colour. The egg has a single large oil globule, and a narrow clean peri-vitelline space. No oil globule was found in the peri-vitelline space at any stage of embryonic development. The advanced embryo with the tail coiled over the head occupies almost the entire peri-vitelline space. It ruptures the egg membrane, hatches out with the tail foremost, and wriggles about with the head end still inside the egg, using the projecting tail portion as a paddle. The lashing movements of the tail help to cast off the half ruptured egg membrane covering the head portion.

The period of incubation in eggs even from the same nest ranges between 24 to 40 hours.

In the laboratory when artificial aeration was not provided mortality was heavy before hatching. When water is allowed to drip slowly in the aquaria, the mortality of eggs and larvæ could be controlled. This seems to indicate that even in the stagnant waters of the pond some little aeration of the egg may be brought about by the slow sinuous movements of the body of the parent to ensure satisfactory hatching.

The hatchling is 4·35 mm. long, considerably pigmented, and has a conspicuous oblong yolk sac from which the head hardly projects forward. It floats at the surface up side down at a slight angle. The eyes are not fully pigmented, and the pectoral buds and mouth have not yet appeared. On the second and third days it is 5·25 mm. and 5·73 mm. long respectively. The yolk sac is almost fully absorbed on the third day when with the well formed mouth the larva begins to feed. The pectoral fins are flap-like. Under natural conditions the growth and differentiation of structures are rapid. The mouth and the tip of the notochord are directed upwards by the 5th and 7th days respectively. The caudal fin has indications of 7–8 rudimentary rays. The colour of the body is reddish, with the eyes golden red and glistening. The characteristic yellow and dark lateral bands along both sides of the body are well indicated. A distinct bright yellow spot has appeared on the dorsum of the head postero-medially, over a spindle-shaped median patch of dark brown pigment extending upto the level of the eyes. On the 9th day the dorsal and anal fins are clearly indicated, almost separate from the caudal fin. Rudimentary rays are present in the dorsal and anal fins; and there are indications of ventral fiin buds almost at the same level as pectorals. Pigmentation has become more pronounced. Within the next two days all the fins are fully formed excepting the ventrals which take two more days to be fully differentiated. Scales appear on the body by the 15th or 16th day.

Post-Larval Growth

(*a*) *In the Laboratory*.—Growth of fry in aquaria was slow. The growth in length of hatchlings fed on live plankton was on an average 8·04 mm. in 15 days, and 12·3 mm. in 36 days.

Growth of fingerlings in aquaria, fed regularly on an ample supply of young stages of forage fish, *Amblypharyngodon mola* and *Esomus danricus*, was as in Table II.

TABLE II

Data on the growth of fingerlings of O. striatus *in the laboratory aquaria (30″×15″×12″) having a water content of 2·4 c.ft.*

Date	No. of specimens	Total length (mm.)		No. of days kept in aquaria	Growth during the period		Remarks
		Range	Aver.		Range	Aver.	
6—3—1946	4	112·0–123·0	119·0	..	..	..	
6—5—1946	4	143·0–148·0	145·25	60	24·0–31·0	26·25	
10—6—1946	4	163·0–180·3	170·3	35	20·0–32·3	25·08	
16—8—1946	only 2	181·0–203·0	192·0	66	16·0–19·7	17·85	
	added 2	148·0–160·0	154·0	..	..	..	
11—9—1946	2	196·0–207·0	201·5	25	4·0–15·0	9·5	
	2	168·0–186·0	177·0	25	20·0–26·0	23·0	poor growth
9—10—1946	4	172·0–209·0	192·0	28	2·0—4·0	3·0	growth very poor
17—12—1946	4	175·0–215·0	194·5	69	nil —6·0	2·5	

During a period of over 9 months, the maximum growth recorded was only 92 mm. The largest specimen, 215 mm. long, was just about one year old after hatching.

(*b*) *In the Natural Pond.*—Samples of early fry (3rd day hatchlings) located in a natural pond were collected from a brood and preserved every alternate day for two weeks. The observations on the natural growth of fry under the protection of the parent fishes as seen from the size and weight of the above collection of fry are detailed in Table III.

The growth of fry under natural conditions is much more rapid than that under laboratory conditions. The period of relatively slow average growth in the first three days after hatching corresponds to the period of subsistence on yolk. The growth is, however particularly rapid from the middle of the second week to the beginning of the third week, when it begins to feed on plankton. By the middle of the third week, growth tends to slow down gradually. This growth in length corresponds to the steady gain in weight from the time the hatchling begins to feed. To begin with, the daily gain in weight is approximately half the weight of the fry at the beginning of the day, increasing to just as much as or even a little over the total weight of the body, by the middle of the second week (Table III, last column). There is a gradual decline after this and by the middle of the third week the daily

TABLE III

Initial growth of early fry of O. striatus *in a natural pond at Azhicode, Cochin*

Date	No. of fry examined	Age (days) after hatching	Total length in mm.			Average growth (mm.)		Aver. wt of fry (gms.)	Average increase in wt. (g.)		Gain in wt. per day.
			Max.	Min.	Aver.	During intervening period	During 24 hours		During intervening period	During 24 hours	Wt. at beginning of day
14-3-1951	7	3	5·85	5·13	5·496	1·146*	0·382	0·0014	..†	..	..
16-3-1951	5	5	7·20	6·30	6·525	1·029	0·514	0·0027	0·0013	0·00065	0·46
18-3-1951	7	7	10·08	7·56	8·73	2·205	1·102	0·0062	0·0031	0·00155	0·57
20-3-1951	7	9	12·4	10·4	11·67	2·94	1·47	0·0148	0·0086	0·0043	0·7
22-3-1951	6	11	16·7	13·8	15·93	4·26	2·13	0·0456	0·0308	0·0154	1·04
27-3-1951	7	16	30·7	25·8	27·78	11·85	2·37	0·2384	0·1928	0·03856	0·84
29-3-1951	7	18	33·8	28·6	31·6	3·82	1·91	0·3446	0·1062	0·0531	0·22

* The average length of hatchling is taken as 4·35 mm. † Weight of the hatchling was not taken.

gain in weight is only about one-fourth the weight of body. Thus, during the first 18 days of the life of the fish, the maximum growth in length and increase in weight are registered from the 11th to the 16th day (*vide* Table III). Though the average growth in length during these 18 days has been just over 6 times the length of the hatchling, the average increase in weight of the three-day old hatchling during the next 15 days has been about 245 times its initial weight. Zooplankton in the pond which consisted mainly of copepods, nauplii and rotifers, was only of very moderate density, and planktonic algæ were extremely rare.

In the natural environment the growth of the post-larvæ up to the fingerling stage is also rapid. Several broods of fry located in the Chetput Farm ponds were sampled at different intervals to ascertain their natural growth. The data gathered are given in Table IV.

TABLE IV

Data on the growth of post-larval specimens of O. striatus *to the fingerling stage in the Chetput Farm, Madras*

Pond	Brood	Date	Total length (mm.)		Intervening period (days)	Average growth (mm.)		Remarks
			Range	Aver.		During intervening period	Per day	
I	1	3—1—1946	38·0—48·0	43·6	..	..	..	
		29—1—1946	59·0—85·0	71·0	26	27·0	1·05	
		6—3—1946	97·0—121·0	110·2	36	39·2	1·08	
	2	24-10—1946	11·6—16·2	14·3	..	..	..	
		29—11—1946	62·0—70·0	65·6	36	51·3	1·42	Aver. wt. 1·8 gms.
III	3	3—1—1946	28·0—32·0	30·3	..	..	..	
	21	21—1 -1946	38·0—46·0	42·5	18	12·2	0·68	Infected by lernæids
		6—3—1946	80·0—89·0	85·2	43	42·7	1·0	
	4	15-10—1946	12·6—16·8	15·1	..	..	..	
		30—11—1946	78·0—99·0	90·8	45	75·7	1·68	Aver. wt. 5·6 gms
		17—12—1946	96·0—109·0	103·5	17	12·7	0·75	Aver. wt. 7·2 mgs.

Growth in the different ponds varies probably with the availability of food. The daily increase in length ranging from 0·75 mm. to 1·68 mm. must be considered to be rapid though it is lower than that recorded in the

TABLE V

Growth, fattening and maturity of fingerlings of O. striatus *stocked in rearing ponds in the Chetput Farm, Madras*

Pond No.	Brood	Date	Total length (mm.)		Aver. wt. per fish (g.)	Intervening period (days)	Aver. growth (mm.)		Sex	Stage of gonad	Remarks
			Range	Aver.			During the period	Per day			
1	2	24-10-1946	11·6—16·2	14·3	..	..	..	..	..	..	
	,,	29-11-1946	62·0—70·0	65·6	1·88	36	51·3	1·42	..	..	
Fingerlings netted and released in rearing ponds on 29-11-1946											
Rearing	,,	21—4—1947	205·0—242·0	230·5	90·0	142	164·9	1·16		Immature	
Pond I	,,	28—5—1947	195·0—273·0	238·7	117·5	37	8·2	0·22	♂ ♀	Immature	
	,,	3—7—1947	238·0—245·0	241·5	..	36	2·8	0·09	♂ ♀	IV	Only 2 specimens netted
	,,	20-11-1947	250·0—270·0	..	..	All fish emaciated, no forage fish present in the pond			♀	V	43 specimens recovered
Rearing	,,	3—8—1947	233·7—317·5	266·2	138·0	246	200·6	0·81	♂ ♀	II–IV	
Pond II	,,	4-10-1947	Fish had bred and fry have grown to 22·0 mm. in length								

post-larval stage. Fry infected by lernæids had their growth adversely affected.

When the fingerling stage is reached the herd instinct of the fry begins to wane and the brood gradually scatters all over the pond. To avoid the possibility of the stock getting mixed up with other broods of fingerlings, segregated rearing was resorted to and the growth followed in two rearing ponds. Pond I had only a limited population of carp minnows introduced along with the murrel fingerlings. In the other pond minnows were already thriving at the time of stocking murrels. Growth of fingerlings was periodically observed by taking measurements and weight of samples. The data gathered are given in Table V.

As the forage fish and murrel fingerlings were introduced together in Pond I there was hardly any time for the former to breed and establish themselves in it. Though natural food such as forage fish was limited in quantity in the pond, it was sufficient to maintain the fairly rapid growth of the murrels over a period of nearly 5 months, after which growth was slow as a result of the diminution in the number of forage fish. Though gonads were maturing in some specimens, there was distinct emaciation and undeveloped gonads in many. Only 66 out of the 300 fingerlings introduced in this pond survived.

As the forage conditions in Pond II were better the murrels had attained a maximum length of 317·5 mm. and weight of half a pound in 8 months after stocking. In another 2 months when the fish were about 11 months old, they started breeding. It is thus clear that under natural conditions *O. striatus* attains sexual maturity and breeds at the end of the first year of its life.

FOOD AND FEEDING HABITS

The predatory habits of *O. striatus* is so well known that no further additions seem possible in respect of its food and feeding habits. Mookerjee *et al.* (*op. cit.*) reported that just after the mouth was formed the larvæ of *O. striatus* fed on protozoa and algæ, while early fry subsisted on planktonic crustacea. The 3-day old hatchlings from the Azhicode pond, however, had not begun feeding, even though the mouth was well formed and functional. On the 5th day the young ones had gorged themselves with eggs and nauplii of copepods, colonial rotifers and other zooplankters, and this was continued on the succeeding days also. The plankton in the pond consisted of predominantly animal forms as a collection made with a No. 21 bolting silk townet showed.

Discussion and Summary

O. striatus breeds almost throughout the year, with a peak immediately preceding and during the monsoon months. Although known as a nest builder amidst weeds in the marginal shallows, it appears capable of breeding even in ponds devoid of all vegetation. Both the parents keep guard over the brood of fry, the larger female being more assiduous in brood care than the male. Under natural conditions sexual maturity is attained in about 11 months after hatching when it is 10″ to 12″ long. The observations of Raj (*op. cit.*), Bhattacharya (*op. cit.*) and Mookerjee *et al.* (*op. cit.*) that *O. striatus* attains sexual maturity when two years old, appear to refer to specimens grown under artificial or adverse conditions. The fecundity of the species seems to increase with the size of the fish. In the normally developing egg the single oil globule does not appear to undergo fragmentation, leaving the perivitelline space clear throughout, although Mookerjee *et al.*, report breaking up of the globule into smaller ones in the perivitelline space, which may happen in faulty preservation of eggs.

Under natural conditions, the larval development is much quicker than what has been recorded hitherto. The 16–20 days stage of Mookerjee *et al.*, which corresponds to the 40 days stage of Willey (*op. cit.*), is attained by the 9th day. The differentiation of structures is even more rapid than in *O. marulius* (Khan, *op. cit.*). Mookerjee *et al.*, state that it attains a maximum length of 320 mm. in two years. A brood of fry hatched in the Madras aquarium pond attained a length of 152·4 mm. in one year and about a foot (304·8 mm.) at the end of the second year (Raj, *op. cit.*). The present author's observations show that growth, during all stages of its life-history, is much more rapid, and that even under limited forage conditions a length of about 320 mm. is attained at the end of the first year. Even in aquaria one-year old specimens attain a maximum length of 215·0 mm. (*vide* Table II).

The observations recorded in the present paper on the feeding habits of the early larvæ of *O. striatus* do not seem to support those of Mookerjee *et al.*, who have concluded that the larvæ feed almost exclusively on algæ and protozoa. In the conditions prevailing in the pond under report there seems to be little possibility of the fry obtaining an exclusive diet of planktonic algæ or protozoans. Available evidence on the natural food of tender fry of the majority of fresh-water fishes shows that an algal diet, at the commencement of alimentation, is perhaps an exception rather than the rule. It has been recently shown (Alikunhi, 1952) in the major Indian carps that an algal diet is an emergency food for the tender fry, and what little algal

diet has been consumed remains undigested and that the fry thrive best on a natural diet of zooplankton. The murrel fry also appear to conform to this general rule.

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ON SOME ABNORMAL FEMALE RECEPTACLES OF *ASTERELLA BLUMEANA* NEES.*

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Asterella blumeana is a member of the Marchantiales assigned to the Rebouliaceæ (Evans, 1939). The species is monœcious, bearing the male receptacle just behind the stalk of the female receptacle. The latter is terminal and long-stalked and usually bears 2–5 involucres. Normally each of the involucres may subtend a sporogonium, enclosed within a characteristic perinath. A normal specimen of *Asterella blumeana* is shown in Fig. 1. According to Kashyap (1929) the thallus, in this species, measures 7–10 mm. × 3 mm. and the stalk of the female receptacle is 8–10 mm. long.

The species is quite common in India, occurring abundantly in the Western Himalayas (Stephani, 1900; Kashyap, 1929); Eastern Himalayas (Mitten, 1861; Chopra, 1928); South India (Chopra, 1938); and Madhya Pradesh (Pandé and Srivastava, 1952).

While examining material of this liverwort, collected by us from Agumbe, in October 1950, we came across some anomalous specimens (Figs. 2, 3, 4 and 5 and Plate Figs. 7 and 8). These are described below.

Specimen 1.—The thallus in this specimen (Fig. 2) measures 4·5 mm. × 3 mm. and bears a stalked female receptacle. The stalk measures 8·5 mm. and carries a convex receptacle, measuring 1·5 mm. × 1 mm. The latter subtends three involucres. In normal plants the growing points of the receptacles give rise to archegonia which, after fertilisation, produce sporophytes. In the specimen under consideration, although the receptacle and involucres have been formed, the former has produced no archegonia, but from one of its growing points a vegetative shoot has developed. This shoot measures 1·6 mm. × 1 mm. and consists of a cylindrical basal and an expanded apical part. As in the normal plants the thalloid portion has pores and air-chambers on its dorsal surface while on its undersurface it bears scales and rhizoids. The other two growing points of the receptacle have, apparently, developed no structures. The second lobe of the normal thallus bears a stalk of the carpocephalum, but no receptacle. Obviously the receptacle got accidentally broken. Each of the two lobes of the main thallus bears a ventral shoot (Fig. 2, *v.i.*).

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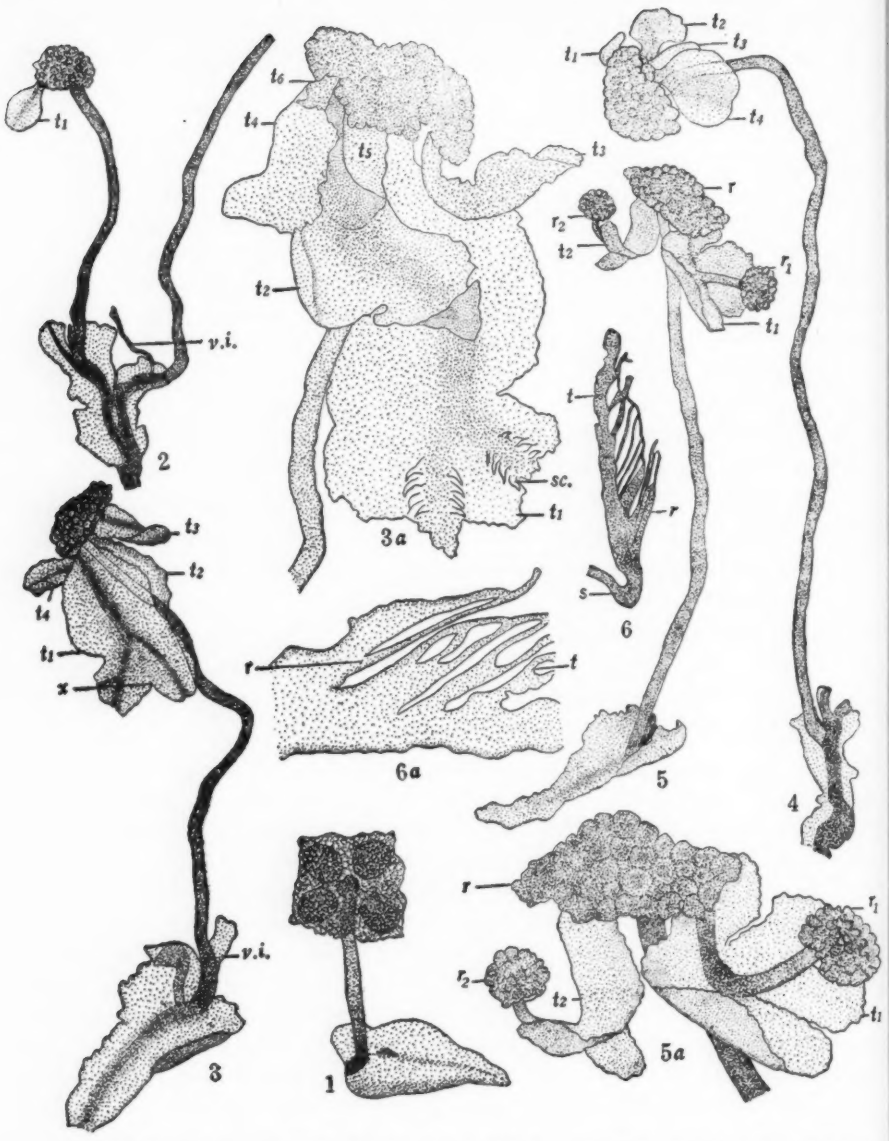

FIGS. 1–6. Fig. 1. Normal thallus, × 5. Fig. 2. Abnormal specimen 1. Note a vegetation thallus (*t*1,) that has developed from the receptacle. *v.i.*, ventral innovation, × 5. Fig. 3. Abnormal specimen 2. Note the vegetative shoots (*t*1–*t*4,) coming out of the involucres, × 5. Fig. 3 *a*. Enlarged view of 3. All the six vegetative shoots *t*1–*t*6, are seen. One of these is seen from the ventral side. *sc.*, scale. × 12. Fig. 4. Abnormal specimen 3. All the

growing points of the receptacle have produced vegetative thalli; four of these (*t*1–*t*4,) can be seen in the figure, × 5. Fig. 5. Abnormal specimen 4. The receptacle shows two vegetative thalli that bear female receptacles. *t*1–*t*2; thalli produced from the two growing points of the receptacle; *r*, primary receptacle; *r*1, *r*2, two secondary receptacles, × 5. Fig. 5 *a*. Enlarged view of 5. *r*, primary receptacle; *r*1, *r*2, secondary receptacles; *t*1, *t*2, vegetative thalli, × 10. Fig. 6. L.S. of the receptacle with l.s. of the vegetative thallus produced from one of the growing points of the receptacle. *r*, receptacle; *t*, vegetative thallus; *s*, stalk, × 12. Fig. 6 *a*. Enlarged view of 6, × 36.

Specimen 2.—In this specimen the thallus measures 6 mm. × 3·5 mm. The receptacle is borne on a long stalk which measures 16·5 mm. and is, apparently, well developed. The receptacle is convex and measures 2·5 mm. × 1·2 mm. and subtends six involucres (Figs. 3 and 3 *a* and Plate Figs. 7 and 7 *a*), all of which are sterile as the receptacle, instead of developing archegonia, has produced vegetative shoots from its growing points (Fig. 3 *a*, *t*1–*t*6). These shoots are of different ages; the youngest one measuring ·6 mm. × ·4 mm. and the oldest 5 mm. × 3 mm.. Two of them have dichotomised in front and the branching can be clearly seen in one of the shoots (Fig. 3, *x*). The structure of the shoots is quite normal, showing the usual type of scales and rhizoids on the undersurface, and normal pores and air-chambers on the dorsal.

Specimen 3.—This specimen is similar to the previous one. The female receptacle is carried on a long stalk which measures 24 mm. and bears six involucres each of which subtends a vegetative shoot arising from the growing point of the receptacle (Fig. 4). The shoots are of different ages. Each of the older shoots among these, consists of a short cylindrical basal portion and an expanded apical portion. The structure of the shoots is of the normal type. On the ventral surface, these bear scales and the two types of rhizoids, while on the dorsal side, they show air-chambers and pores as in normal plants. Two of the shoots show distinct dichotomy at the apex.

Specimen. 4.—This specimen (Figs. 5, 5 *a* and Plate Fig. 8) appears to be somewhat more interesting. The thallus measures 6 mm. × 2·5 mm. The female receptacle is stalked, the stalk measuring 16·3 mm. while the receptacle measures 3·5 mm. × 1 mm. It is convex and bears two involucres on the two opposite sides of the receptacle. Both the involucres are sterile. Each growing point of the receptacle, in these involucres, has developed a vegetative shoot. The latter consists of a very short stalk-like basal portion, with which it is attached to the receptacle, and an expanded apical portion. One of the shoots is slightly older than the other. On the dorsal surface of these, abnormally developed thalli, there are pores and air-chambers, while their ventral surface bears rhizoids and scales. Both the vegetative shoots, while yet attached to the carpocephalum, have developed apical female

receptacles (Fig. 5 and Plate Fig. 8) as in the normal plants. The stalk of the older one measures 1·5 mm. and the receptacle itself 1·25 mm. × 1 mm. In this receptacle there are indications of four rudimentary involucres. Behind the stalk of the female receptacle there is a male receptacle but no antheridia have been produced.

The second abnormal shoot is comparatively younger. The stalk of the receptacle, in this case, measures 1 mm. while the receptacle is 1 mm. × ·9 mm. In this receptacle, also, there are indications of the formation of four involucres. The tissue of the receptacle, in both the cases, bears barrel-shaped pores. Like specimen one, this specimen, too, bears two ventral innovations, arising from near the apex. One of the ventral shoots has innovated again.

CONCLUDING REMARKS

As stated by Müller (1906–11, pp. 107–108), Lindberg (1875) and later on Klien (1881), noticed adventitious shoots coming out from the inflorescence and rhizoidal furrow of the stalk of *Dumortiera* and *Marchantia* (see Müller, *l. c.*, p. 108, Fig. 84), which under favourable conditions grow out into new plants.

Peissel (1925), as stated by Bergdolt (1926), notes abnormal female receptacles in *Asterella blumeana* in which vegetative shoots develop from the receptacle and its stalk. Bergdolt (*l.c.*) describes and figures (Bergdolt's Fig. 105, p. 61) a vegetative shoot arising from the growing point of one of the involucre of *Sauteria alpina*. Kashyap (1929) observes that in *Marchantia palmata* vegetative shoots develop from the carpocephalum and its peduncle. The cases described by us in *Asterella blumeana* are more or less of the same type. It is unfortunate that the original paper by Peissel was not available for comparison.

Srinivasan (1939) observes that in *Marchantia palmata* proliferations arise from the female receptacles in the form of small narrow lobes which produce antheridia directly. The proliferations noticed by us in *Asterella blumeana* grow out into normal vegetative thalli, which may remain sterile or may produce male and female receptacles (specimen four, Figs. 5 and 5 *a* and Plate Fig. 8) as in normal plants.

In all our specimens, the female receptacles are comparatively long stalked (compare Figs. 1, 2, 3, 4 and 5 and Plate Fig. 7) and, apparently, follow the normal course of development and form involucres all of which, obviously, remain sterile and some even without any further noticeable growth but others produce vegetative shoots from the growing points of the receptacle (Figs. 2, 3, 4 and 5). The number of growing points of the

receptacles, functioning in this way, varies. In two of the cases all the six growing points of the receptacles, the maximum number observed by us in this species, produce normal thalli while in other cases one, two or three of these may function in this way. The structure of the thalli, so produced, is, in all respects, similar to that of the ordinary thallus (Fig. 5). These shoots originate from the growing points of the receptacles and are organically connected with them (Figs. 6 and 6 *a* and Plate Figs. 9 and 9 *a*). It is thus obvious that the growing points of the receptacles, which ordinarily produce archegonia, have developed vegetative thalli. In all the specimens, examined by us, the shoots are borne on the apparently ventral side of the receptacle but, as we know from the history of the development of the receptacle and sex organs in *Asterella* and the allied genera, these originate on the dorsal surface. Their ventral position is due to subsequent downward shifting. For want of material a detailed developmental study of the vegetative shoots could not be followed. Each shoot consists of an expanded apical and a thick cylindrical basal part. In the specimens where several thalli are produced these are of different ages (Figs. 3 and 3 *a*). The shoots, if detached would, undoubtedly, continue to grow and develop into independent plants.

The culminating stage of modification is shown by specimen 4 in which the thalli, produced from the receptacle, while yet attached to it have developed female receptacles, apparently, of the normal type except that these bear no archegonia.

The sterile shoots developed in the specimens, described here, show normal structure. A careful examination of microtome sections (Fig. 6) of one such specimen, showed no infection either by a fungus or any other pathogen. Apparently this is not a pathological phenomenon.

In liverworts, as a rule, vegetative reproduction plays an important part and, in many species, its role is even more important than that of the sexual process and, not unoften, this tendency has resulted in the total suppression of the sexual organs and their replacement by sterile structures. Apparently the conditions, under which liverworts grow, are often more favourable for the vegetative growth, and this possibly may have been the cause of degeneration and even elimination of the sexual organs and their replacement by the sterile shoots. The abnormal specimens of *Asterella blumeana*, described here, would support such an assumption. The shoots thus produced serve as organs of vegetative propagation but under xerophytic or otherwise unfavourable conditions, these would, undoubtedly, become condensed and tuberous and function as perennating organs.

ACKNOWLEDGEMENT

This paper presents a part of the work done under the Research Scheme, "Study of the Hepatic Vegetation of India," sanctioned by the Scientific Research Committee, Uttar Pradesh, and the senior author sincerely thanks the authorities concerned for grant of funds which has facilitated this work.

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EXPLANATION OF PLATE

FIG. 7. Abnormal specimen 2. Photographed from the ventral side. Note the vegetative thalli coming out from the receptacle, × 5.

FIG. 7 *a*. Enlarged photograph to show the vegetative shoot. Note the air pores on the thallus, × 14.

FIG. 8. Abnormal specimen 4. Photograph shows the vegetative thalli that have developed from the receptacle. Each vegetative shoot has developed a female receptacle, × 12

FIG. 9. Microphotograph of the L.S. of receptacle bearing vegetative thallus. *th*, thallus; *r*, receptacle, × 24.

FIG. 9 *a*. Same as above. *th*, thallus; *r*, receptacle, × 72.

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* Original publication was not seen.

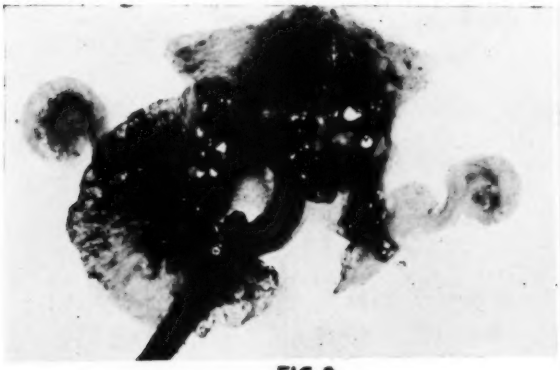

FIG. 8

FIG. 7

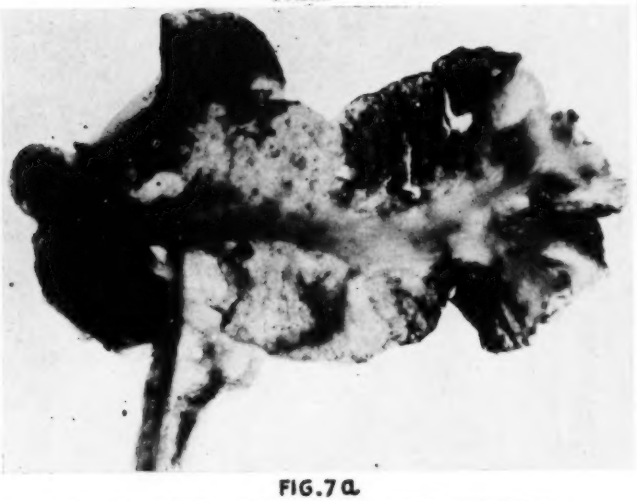

FIG. 7a

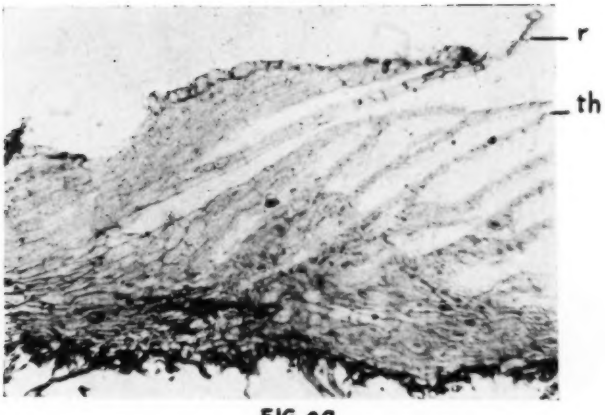

FIG. 9a

FIG. 9

FUNGI IMPERFECTI FROM MADRAS—V

CURVULARIA

By C. V. Subramanian

(*University Botany Laboratory, Madras*)

Received March 30, 1953

(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

This paper is a systematic account of some species of *Curvularia* collected by me recently. Two new species, *C. indica* and *C. palmarum* are described. Eight other species are recorded: *C. maculans*, *C. lunata*, *C. pallescens*, *C. andropogonis*, *C. trifolii*, *C. inæqualis*, *C. falcata* and *C. uncinata*. Of these, all species except *C. lunata* are new records for India. The only other species known from this country is *C. penniseti* (Mitra) Boedijn.

19. *Curvularia maculans* (Bancroft) Boedijn, in *Bull. Jard. bot. Buitenz., Ser. III*, 1933, **13**: 125, ic.

Basinym: *Spondylocadium maculans* Bancroft (1913); see also Mason E. W., 1928, *Annotated Account of the Fungi Received at the Imperial Bureau of Mycology*, List II, Fascicle 1, p. 5.

Conidiophores brown, simple, unbranched, erect, straight or bent, septate, up to 5 μ broad, of variable length. Conidia borne in spirals towards the tip of the conidiophore, brown, barrel-shaped, broadest in the middle, 3-septate, the two middle cells concolorous with each other and darker than the apical and basal cells, the apical and the basal cells with broadly rounded outline, the basal cell with a distinct scar, $22 \times 12 \cdot 8$ $(17\text{–}26 \times 11\text{–}16)$ μ.

Two collections have been seen: on dead culms of *Scirpus* sp., Poonamallee (Chingleput District, Madras State), 24-2-1953, coll. C. V. S., Herb. M.U.B.L. No. 848–III; on dead leaf-sheath of *Musa paradisiaca* L., Athur Farm (Chingleput District, Madras State), 2-12-1951, coll. C. V. S., Herb. M.U.B.L. No. 650.

Both collections agree well with the description given by Boedijn (1933).

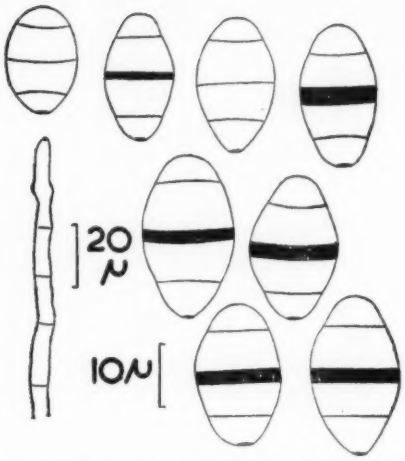

FIG. 1. *Curvularia maculans* : from Herb. M.U.B.L. No. 848–III.

20. *C. lunata* (Wakker) Boedijn, 1933, in *Bull. Jard. bot. Buitenz.*, Ser. III, **13**: 127, ic.; Groves, J. W. and Skolko, A. J., 1945, *Canad. J. Res.*, C, **23**: 101, ic.

Basinym: *Acrothecium lunatum* Wakker (1898). Saccardo, *Syll. Fung.*, **14**: 1089, 1899; see also Mason, E. W., 1928, *Annotated Account of the Fungi Received at the Imperial Bureau of Mycology*, List II, Fascicle 1, p. 5.

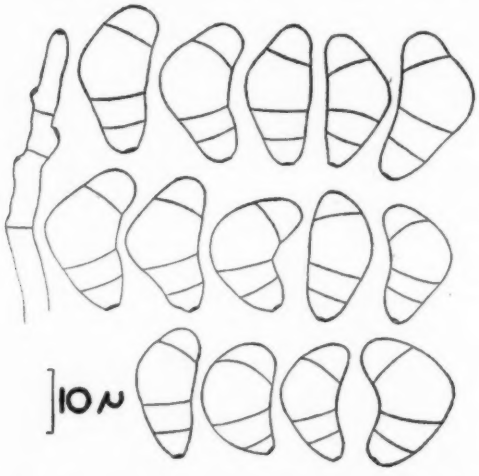

FIG. 2. *Curvularia lunata* : from Herb. M.U.B.L. No. 174.

Conidiophores brown except towards the tip where they are paler, simple, unbranched, septate, 3–6 μ broad, of variable length, geniculate towards the tip. Conidia boat-shaped, brown, 3-septate, the third cell from the base conspicuously larger, broader and darker than the others, curved or sometimes straight, each with a subhyaline, rounded apical cell, and a subhyaline, somewhat obconical basal cell which bears a scar indicating the point of attachment to the conidiophore, 21×10 ($17–24 \times 8–12$) μ.

Three collections have been examined: on dead culms of grass, Marina, opposite the University Buildings, Madras, 3–3–1951, coll. C. V. S., Herb. M.U.B.L. No. 165; on dead stubble of *Eleusine coracana* Gaertn., Vandalur (Chingleput District, Madras State), 6–4–1951, coll. C. V. S., Herb. M.U.B.L. No. 174; on dead leaf of *Borassus flabellifer* L., University Botany Laboratory campus, Madras, 23–4–1951, coll. C. V. S., Herb. M.U.B.L. No. 218.

All the three collections agree well with the description given by Boedijn (1933).

21. *C. pallescens* Boedijn, in *Bull. Jard. bot. Buitenz.*, Ser. III, 1933, **13**: 127, ic.; Groves, J. W. and Skolko, A. J., *Canad. J. Res.*, C, 1945, **23**: 102, ic.

Conidiophores brown, simple, unbranched, septate, with geniculate tip, 3·2–5·6 μ broad, of variable length. Conidia produced in spirals

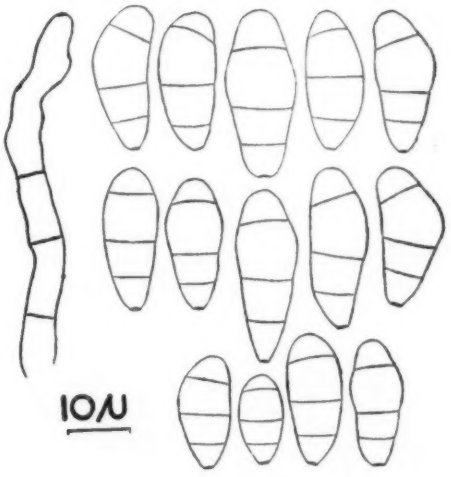

FIG. 3. *Curvularia pallescens* : from Herb. M.U.B.L. No. 91-II.

towards the tip of the conidiophore, pale brown, somewhat cylindrical, mostly straight or only with a slight curvature, 3-septate, the middle two cells slightly darker in colour than the basal and apical cells, apical cell hyaline and with rounded tip, the basal cell subhyaline, shaped like a crucible and with a scar at the base indicating the point of attachment with the conidiophore, 22×9 $(14–28\times6–12)\,\mu$.

The majority of the conidia lack a distinct curvature, although the third cell from the base may be slightly larger than the others. The middle two cells are pale brown and concolorous with each other, whereas the basal and the apical cells are subhyaline.

Only one collection has been seen: on dead palm leaf, University Botany Laboratory campus, Madras, 22–2–1951, coll. C. V. S., Herb. M.U.B.L. No. 91–II.

22. *C. andropogonis* (Zimmermann) Boedijn, in *Bull. Jard. bot. Buitenz.*, Ser. III, 1933, **13**: 128, ic.

Basinym: *Napicladium andropogonis* Zimmermann (1902). Saccardo, *Syll. Fung.*, 1906, **18**: 594.; see also Mason, E. W., 1928, *Annotated Account*

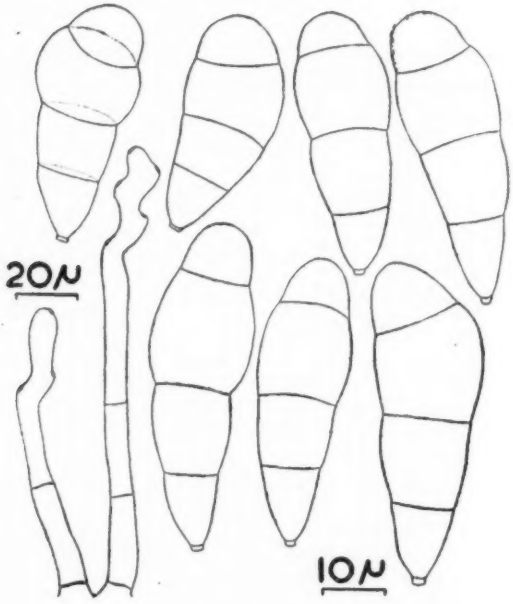

FIG. 4. *Curvularia andropogonis* : from Herb. M.U.B.L. No. 94.

of the Fungi Received at the Imperial Bureau of Mycology, List II, Fascicle 1, p. 5.

Conidiophores brown, simple, unbranched, septate, geniculate and paler towards the tip, 3–4 μ broad, of variable length. Conidia pale brown in colour, somewhat ellipsoidal, 3-septate, constricted at the septa, the third cell from the base larger, broader and darker than the others, the apical cell with a broadly rounded tip, the basal cell elongate-obconical and with a prominent, small, short, hyaline pedicel at the base where it is attached to the conidiophore, $45 \times 15 \cdot 5$ $(40–53 \times 14–18)\ \mu$.

Only one collection has been examined: on dead leaves of *Dichanthium annulatum* Stapf., Agri-Horticultural Society's Gardens, Teynampet, Madras 25-2-1951, coll. C. V. S., Herb. M.U.B.L. No. 94.

The fungus agrees well with the description given by Boedijn (1933).

23. *C. trifolii* (Kauffman) Boedijn, in *Bull. Jard. bot. Buitenz.*, Ser. III, 1933, **13**: 128; Groves, J. W. and Skolko, A. J., *Canad. J. Res.*, C, 1945, **23**: 101, ic.

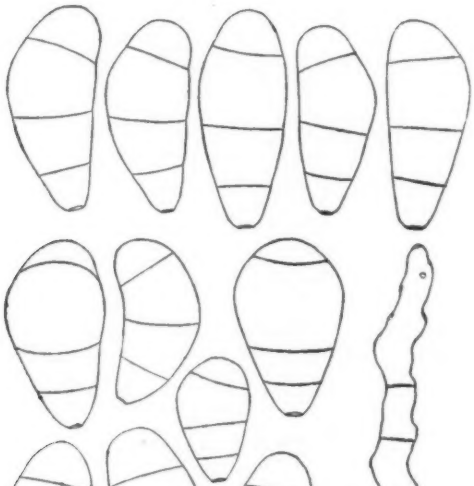

FIG. 5. *Curvularia trifolii* : from Herb. M.U.B.L. No. 851-I.

Basinym: *Brachysporium trifolii* Kauffman (1920). Saccardo, *Syll. Fung.*, 1931, **25**: 835; see also Mason, E. W., 1928, *Annotated Account of the Fungi Received at the Imperial Bureau of Mycology*, List II, Fascicle 1, p. 5.

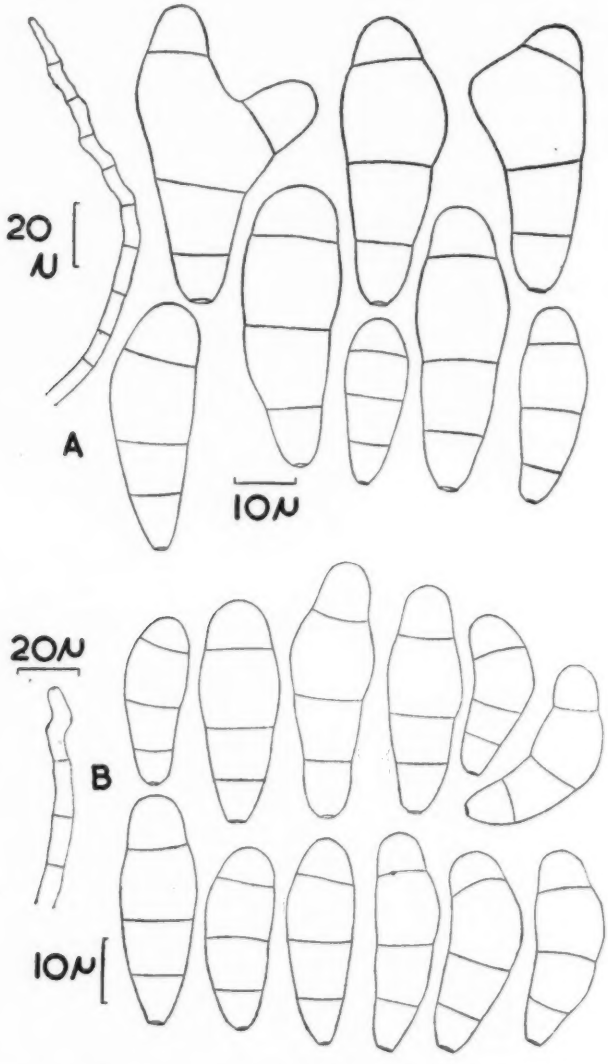

FIG. 6. *Curvularia indica* : A, from type specimen, Herb. M.U.B.L. No. 851-II; B, from Herb. M.U.B.L. No. 848-II.

Conidiophores brown, simple, unbranched, septate, geniculate and paler at the tip, 3·3–4·8 μ in diameter, of variable length. Conidia brown, unequally ventricose-fusiform, curved or sometimes somewhat straight, 3-septate, not constricted at the septa, the third cell from the base larger, broader and darker than the others, the apical cell with a smoothly rounded tip, the basal cell crucible-shaped with a distinct scar at the base indicating the point of attachment to the conidiophore, 28·8×13·4 (19–36×11–16) μ.

Only one collection has been seen: on dead culms of *Scirpus* sp., Poonamallee (Chingleput District, Madras State), 24–2–1953, coll. C. V. S., Herb. M.U.B.L. No. 851–I.

The fungus collected by me comes nearest to *C. trifolii*, as re-described by Groves and Skolko (1945, pp. 101–102, ic.) after examining the type specimen. The measurements of the conidia of the type specimen as given by Groves and Skolko are: 25–35–(38)×11–15 μ, and the measurements of the conidia in my collection are similar. It is true that my fungus has a substratum different from that on which the species was originally described, but this is of not much importance in a genus like *Curvularia*, species of which have been reported on diverse substrata.

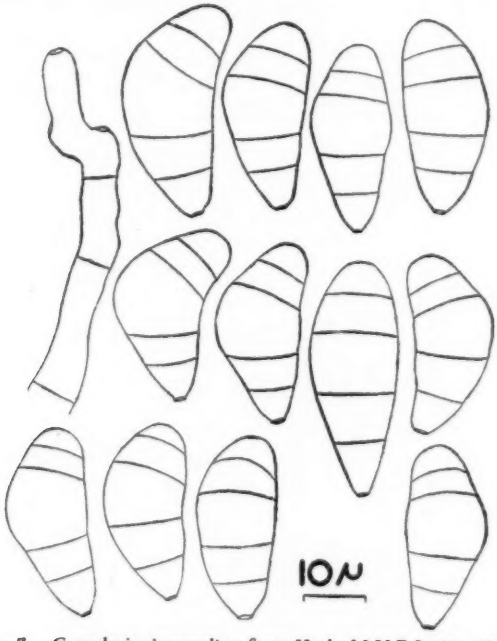

FIG. 7. *Curvularia inæqualis* : from Herb. M.U.B.L. No. 104.

24. **Curvularia indica** Subramanian sp. nov.

Conidiophori simplices, brunnei, erecti, recti vel curvati, septati, 4·8–8·3 μ lati, longitudinis variabilis. Conidia spiraliter infixa apicibus conidiophororum, ellipsoidea, ut plurimum recta, raro curvata, brunnea, 3-septata, tenuiter constricta ad septa, vel nullo modo constricta, cellula tertia supra basim ampliori et latiori cæteris, cellula apicali lævi, rotundata ad apicem, cellula basali catino simili, insignita cicatrice infra, 24–47 × 8–16 μ.

Habitat in culmis emortuis *Scirpi* sp., Poonamallee (Chingleput District, Madras), 24–2–1953, legit. C. V. S., Herb. M.U.B.L. Nos. 851–II (Typus), 848–II.

C. indica Subramanian sp. nov.

Conidiophores brown, simple, erect, straight or bent, septate, 4·8–8·3 μ broad, of variable length. Conidia borne in spirals towards tip of the conidiophore, ellipsoidal, mostly straight, seldom curved, brown, 3-septate, slightly constricted at the septa or not at all, the third cell from the base larger and broader than others, the apical cell with a smoothly rounded tip, the basal cell crucible-shaped with a scar at the base, 24–47 × 8–16 μ.

Habit.: on dead culms of *Scirpus* sp., Poonamallee (Chingleput District, Madras State), 24–2–1953, coll. C. V. S., Herb. M.U.B.L. Nos. 851–II (Type), 848–II.

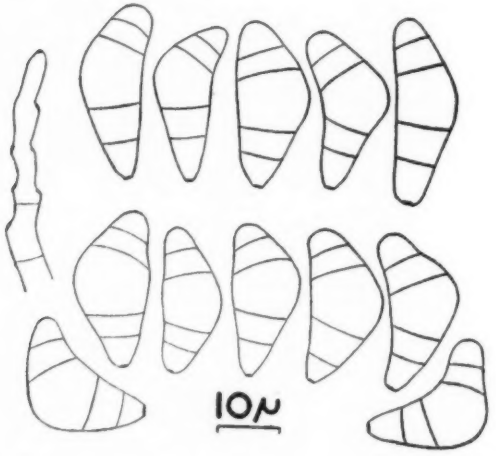

FIG. 8. *Curvularia falcata* : from Herb. M.U.B.L. No. 91–III.

The measurements of the conidia in the two collections are:

Herb. M.U.B.L. No. 851–II: $39 \times 13 \cdot 1$ (25–47 × 9–16) μ;

„ 848–II: $33 \times 11 \cdot 4$ (24–40 × 8–13) μ.

My fungus has 3-septate spores and is easily placed in the Lunata group. Amongst the seven known species of this group, it comes nearest to *C. stapeliæ* (du Plessis) Hughes and du Plessis (Hughes, 1951, p. 28), originally described from South Africa by du Plessis as *Triposporium stapeliæ* du Plessis. The measurements of conidia in *C. stapeliæ* are given by Hughes (1951) as: 26–41 × 10–15 μ. The size of the conidia of my fungus falls largely within the range covered by *C. stapeliæ*, despite the fact that a few conidia were longer (up to 47 μ). The conidia of my fungus, however, differ from those of *C. stapeliæ* in the following features: (i) the conidia are not conspicuously curved as in *C. stapeliæ*, but the majority of them are straight; (ii) in the case of conidia of *C. stapeliæ* the third cell from the base alone is the darkest, but in my fungus the two middle cells are concolorous with each other and are darker than the apical and the basal cells.

On the basis of these differences, my fungus has been described as a new species.

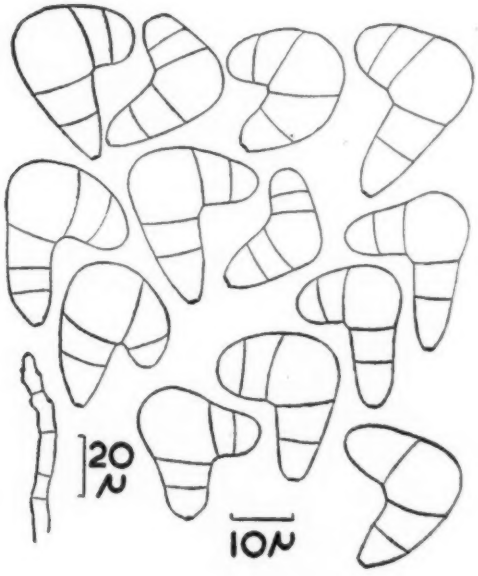

FIG. 9. *Curvularia uncinata* : from Herb. M.U.B.L. No. 848–I.

25. *C. inæqualis* (Shear) Boedijn, in *Bull. Jard. bot. Buitenz.*, Ser. III, 1933, **13**: 129; Groves, J. W. and Skolko, A. J., *Canad. J. Res.*, C, 1945, **23**: 97, ic.

Basinym: *Helminthosporium inæquale* Shear, in *Bull. Torrey bot. Cl.*, 1907, **34**: 307. Saccardo, *Syll. Fung.*, 1913, **22**: 1391; see also Mason, E. W., 1928, *Annotated Account of the Fungi Received at the Imperial Bureau of Mycology*, List II, Fascicle 1, p. 5.

Conidiophores brown, simple, unbranched, septate, up to 5 μ broad, of variable length, geniculate towards the tip. Conidia borne in spirals, brown, 4-septate, the middle cell the broadest and the darkest, lower and upper

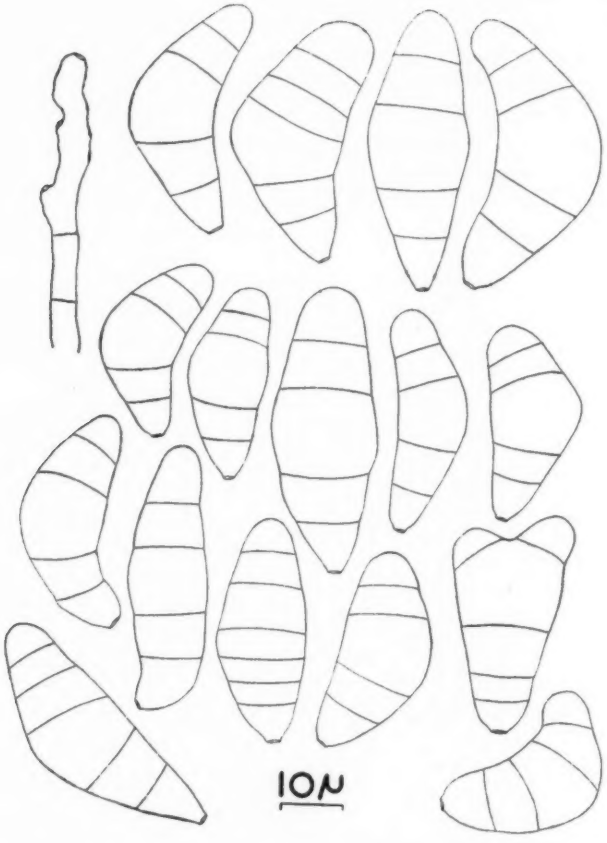

FIG. 10. *Curvularia palmarum* : from type specimen, Herb. M.U.B.L. No. 132.

cells progressively paler in colour, the two basal cells longer than the two apical ones, apical cell with a smoothly and broadly rounded tip, the basal cell obconical with a scar indicating the point of attachment to the conidiophore, $29{\cdot}4\times13{\cdot}6$ $(27\text{–}37\times11\text{–}18)\,\mu$.

Only one collection has been seen: on dead leaves of palm (?*Cocos nucifera* L.), Velacheri (near Guindy, Madras), 25–2–1951, coll. C. V. S., Herb. M.U.B.L. No. 104.

This species, originally placed in the Lunata group by Boedijn (1933), has been transferred to the Geniculata group by Groves and Skolko (1945) after a study of the type specimen, and my fungus agrees with the description given by Groves and Skolko.

26. *C. falcata* (Tehon) Boedijn (as *C. flacata*), in *Bull. Jard. bot. Buitenz.*, Ser. III, 1933, **13**: 130; Groves, J. W. and Skolko, A. J., *Canad. J. Res.*, C, 1945, **23**: 99, ic.

Basinym: *Acrothecium falcatum* Tehon (as *A. flacatum*), 1919, in *Bot. Gaz.*, **67**: 509, ic.; Saccardo, *Syll. Fung.*, 1931, **25**: 813; see also Mason, E. W., 1928, *Annotated Account of the Fungi Received at the Imperial Bureau of Mycology*, List II, Fascicle 1, p. 5.

Conidiophores brown, simple, unbranched, septate, 3–5 μ broad, variable in length, geniculate towards the tip. Conidia brown, unequally ventricose-fusiform, conspicuously curved, rarely otherwise, 4-septate, the middle cell the broadest and the darkest of all, the lower and the upper cells becoming progressively paler, the apical cell somewhat conical and with a rounded tip, the basal cell obconical and narrowing below to a basal scar indicating the point of attachment to the conidiophore, $25\times10{\cdot}4$ $(22\text{–}29\times9\text{–}12)\,\mu$.

Only one collection has been seen: on dead palm leaf, University Botany Laboratory campus, Madras, 22–2–1951, coll. C. V. S., Herb. M.U.B.L. No. 91–III.

This fungus comes nearest to *C. falcata* amongst the species belonging to the Geniculata group. A re-description of the species based on a study of the type specimen is given by Groves and Skolko (1945) and the conidial measurements given by them are: (22)–24–28–(31)$\times$(10)–12–13–(14) μ.

27. *C. uncinata* Bugnicourt, in *Rev. gén. Bot.*, 1950, **57**: 73 (photographs given).

Conidiophores brown, simple, unbranched, septate, up to 5 μ broad, of variable length. Conidia brown, 4-septate, the middle cell the largest, broadest and darkest of all, conspicuously bent at an angle of 90° or more

and hence shaped like a hammer or markedly uncinate, the lower and the upper two cells becoming progressively paler, the two apical cells shorter than the two basal cells, the apical cell with a smoothly rounded tip, the basal cell somewhat obconical and with a prominent scar at the base indicating the point of attachment to the conidiophore, $24 \times 12 \cdot 6$ ($20–29 \times 9–15$) μ.

Only one collection has been seen: on dead culms of *Scirpus* sp., Poonamallee (Chingleput District, Madras State), 24–2–1953, coll. C. V. S., Herb. M.U.B.L. No. 848–I.

This species was first described on *Oryza sativa* L. from Indo-China by Bugnicourt (1950) and my fungus agrees well with the description given by Bugnicourt. Bugnicourt's description indicates the following range in size for the conidia: $18–32 \times 8 \cdot 6–12 \cdot 7$ μ. In my fungus some conidia are up to 15 μ broad. The middle cell in my fungus is somewhat verrucose and, although Bugnicourt's description does not indicate any such peculiarity for his fungus, his photograph of the spores of the species suggests a somewhat verrucose middle cell. I have, therefore, placed my fungus in *C. uncinata*.

28. **Curvularia palmarum** Subramanian sp. nov.

Conidiophori brunnei, simplices, erecti, recti vel curvati, septati, 3–5 μ lati, variabilis longitudinis. Conidia spiraliter infixa apicibus conidiophororum, fusiformia vel falcata, vel inæqualiter fusiformi-ventricosa, brunnea, 4-septata, cellula media omnium maxima, latissima atque obscurissima, cellulis superioribus atque inferioribus pallidioribus atque angustioribus, cellula apicali lævi atque rotundata ad apicem, cellula basali late rotundata vel obconica, insignita cicatrice distincta, $37 \cdot 6 \times 14 \cdot 2$ ($27–48 \times 11–20$) μ.

Habitat in foliis emortuis *Cocos nuciferæ* L., Luz, Mylapore, Madras, 6–3–1951, legit C. V. S. (Herb. M.U.B.L. No. 132).

Curvularia palmarum Subramanian sp. nov.

Conidiophores brown, simple, erect, straight or bent, septate, 3–5 μ broad, of variable length. Conidia borne in spirals towards tip of the conidiophore, fusiform or falcate or unequally fusiform-ventricose, brown, 4-septate, the middle cell the largest, broadest and darkest, the upper and lower cells paler and narrower, apical cell with smoothly rounded tip, basal cell broadly rounded or obconical with a distinct scar, $37 \cdot 6 \times 14 \cdot 2$ ($27–48 \times 11–20$) μ.

Habit.: on dead leaf of *Cocos nucifera* L., Luz, Mylapore, Madras, 6-3-1951, coll. C. V. S., Herb. M.U.B.L. No. 132.

This fungus is easily placed in the Geniculata group. The measurements of the conidia are different from those of any species in this group so far known and I, therefore, consider my fungus as a new species.

I am grateful to Prof. T. S. Sadasivan for critically reading the manuscript and to Prof. H. Santapau for the Latin diagnoses of the two new species.

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SWARMING OF *NOCTILUCA* IN THE PALK BAY AND ITS EFFECT ON THE 'CHOODAI' FISHERY, WITH A NOTE ON THE POSSIBLE USE OF *NOCTILUCA* AS AN INDICATOR SPECIES[1]

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An unusual occurrence of *Noctiluca miliaris* Suriray in great numbers in the inshore waters of the Palk Bay off Mandapam was noticed in April 1952. Their first appearance was on the 14th when in one c.c. of the standardized sample of plankton[2] there were only 25 *Noctiluca*. Soon they showed signs of rapid increase and by April 18 their number in one c.c. reached the order of 3,300. From then on they continued to be present in this area in swarms until July except on three days—May 5, 30 and June 2 when there was an appreciable decline in their numbers being 52, 40 and 60 respectively per c.c. When these occur in enormous numbers a discolouration of the water is clearly visible from the shores showing generally shades of red. Locally the *Noctiluca* which swarmed did not have a pink or red colour as observed along the west coast of India or off Madras, but had a bright green tint because of the presence of numerous actively moving green flagellates in them and they produced a green colouration of the surface water. Delsman (1939) noted that: "A peculiarity of the tropical *Noctiluca* is the presence of a large number of small green flagellates in the spaceous vacuoles, as first stated by the Weber's who, however, did not observe living material. If we do so, we find that these small green organisms of which hundreds, perhaps even thousands, are present in each *Noctiluca*-cell do not be still there but are swimming vigorously about in the fluid which fills the spaceous vacuoles."

Aiyar (1935) was probably the first to record the widespread mortality among fishes in Indian waters caused by the swarming of *Noctiluca*. In

[1] Published with the permission of the Chief Research Officer, Central Marine Fisheries Research Station, Mandapam Camp, South India.

[2] The entire plankton sample collected during a fifteen-minute horizontal haul was made up to 250 c.c. and after thoroughly mixing it a sub-sample of 1 c.c. taken from this was used for counting the number of *Noctiluca* and other organisms.

the opinion of Devanesan (1942) *Noctiluca* is a competitor for the food of oil-sardines as both feed on diatoms such as *Coscinodiscus, Nitzschia* and *Fragillaria* and the abundance of the inedible *Noctiluca* may have a bearing on the scarcity of oil-sardines along the west coast of India at certain seasons. He further adds that the spawning mackerels probably select areas where the *Noctiluca* abound for laying eggs as fish feeding on fish-eggs would loathe to enter such areas. Panikkar (1949) remarked: "It is known that 'red water' phenomena are inimical to fisheries because shoaling fish do not frequent such patches, and on the Indian coasts the fishermen have learnt to avoid such patches." More recently abrupt set-backs in the fisheries of the Malabar and South Kanara coasts have been reported by Bhimachar and George (1950). They remark: "The immediate effect of the occurrence of the 'red water' or slimy water phenomena was a steep fall in the fish catches over a long stretch of the coastal waters. It is clear from our observations and enquiries that the bulk of commercial fishes—particularly the shoaling species, the chief among them being the mackerels and allied forms, sardines and anchovies—did not appear in such waters. There was however no severe mortality among fishes. It would appear that the physico-chemical conditions of the water thus affected were in some way unfavourable for fish to live that they completely avoided such areas." Further, they add: "It appears clear from the foregoing observations that the severe set-back in the fishery over a long stretch of coastal waters in the Malabar and South Kanara Districts during October 1948 was mainly due to the putrefaction of the superabundant *Noctiluca* population, giving rise to thick masses of slimy substance occurring in a state of suspension in the coastal waters. The most characteristic feature of the phenomenon was the extensive slimy masses of dead and decaying *Noctiluca* mixed partly with the fine clay of the mud banks characteristic of the area."

As for the causes responsible for these sudden outbursts it is not possible now to give satisfactory explanations and as Galtsoff (1948) has pointed out: "A thorough knowledge of the ecological conditions preceding the outbreak of red tide, as well as those existing during and after its maximum development are necessary for the understanding of the problem." However, it may be mentioned that although there were no violent fluctuations in the concentration of phosphates, nitrates and pH from January to July 1952, the monthly average values for nitrates were clearly of a lower order during May, June and July compared to the preceding months and also in relation to the values obtained for June-July of the previous year (Table I). Also, there were indications of an increase in the silicate contents of the sea water (although within the normal range of silicate in sea water, Ref. Robinson and

Thompson, 1948) at the time when there were swarms of these dinoflagellates. Ketchum and Keen (1948) observed unusually high phosphorus concentration in the Florida 'Red tide' sea-water; the total phosphorus content varied from 4·9 to 20·4 microgram atoms per litre which was approximately two and a half to ten times the normal expected concentration. The total phosphorus content estimated during the swarming of *Noctiluca* ranged between 0·80 to 1·29 microgram atoms per litre and at other times the samples taken at the same place contained 0·77 to 1·03 microgram atoms per litre, consequently the values were within the normal range to be expected in sea water. The salinity showed a steady rise from January to July 1952 and compared to the previous year, it was higher in May-June and lower in July (Table I).

TABLE I

Hydrological data on the surface waters of the Palk Bay. The values are averages for the months and blanks indicate the lack of data

Month	1951					1952				
	Salinity °/₀₀	pH	Phosphate mg/m³	Nitrate mg./m.³	Oxygen saturation %	Salinity °/₀₀	pH	Phosphate mg./m.³	Nitrate mg./m.³	Oxygen saturation %
Jan.	..	..	..	..	..	28·52	8·45	5·20	61·75	74·60
Feb.	..	..	..	..	..	29·58	8·40	6·36	51·00	63·22
March	..	..	..	..	..	30·89	8·40	5·55	53·57	65·75
April	..	..	..	..	..	32·00	8·40	5·42	59·42	66·25
May	32·16	8·46	5·23	38·07	64·46	33·03	8·35	4·78	41·85	63·57
June	33·49	8·46	8·00	66·00	..	33·78	8·41	8·07	39·33	77·33
July	34·50	8·48	..	71·00	..	33·80	8·40	6·45	42·00	65·25

The occurrence in good numbers of *Creseis acicula* during April seems to be of interest in this connection. They first appeared on April 8 and in all subsequent plankton samples of April they were present. By about the second week of May they almost completely disappeared. Last year during May they were not present in any of the plankton samples and no data are available for April 1951. Whether the appearance of these and the higher salinity recorded this year for May and June indicate an influx of water from elsewhere into this area, during the period under consideration, is a possibility that cannot be overlooked. It may also be mentioned that *Evadne tergestina* occurred in very small numbers during last year only in July but

this year they were common during May and continued to be present up to the beginning of July although in fewer numbers. If these indicate an incursion of water masses from outside there is the possibility that the *Noctiluca* might have been brought into this area. However, no significant differences in the species composition of the diatoms which were present during 1951 and 1952 were noticed although the diatom population level was considerably lower in May, June and July 1952. The copepods on the other hand were higher during May-June and lower during July 1952 as compared to 1951 (Table II). But it must be noted that though the monthly average figures for copepods were higher during May-June there was an appreciable decimation of copepods on the days when the intensity of *Noctiluca* population was high.

TABLE II

Average number of diatoms and copepods per c.c.

Month	1951		1952	
	Diatoms	Copepods	Diatoms	Copepods
April ..	..	..	10216	171
May ..	85604	163	25174	323
June ..	69394	144	56879	311
July ..	56609	352	495	258

The swarming of *Noctiluca* was not accompanied by their death, decay and subsequent formation of a slimy substance as noticed by Bhimachar and George *op. cit.*, and there was no appreciable reduction in the oxygen saturation, it being of the usual order (Table I). During this period the range of saturation varied between 91% in June 2 and 44% in July 11.

Since there has been reports of the swarming of *Noctiluca* causing heavy mortality amongst marine fauna the present occurrence of these in great numbers was watched with interest for its possible effects on the local fauna especially fishes. Curiously enough contrary to the observations made along the coast of Madras, no cases of mass mortality caused by the phenomenon among marine fauna were noticed but there was a definite effect on the local fish and fisheries.

During the period from the middle of March to the middle of July there is a very lucrative fishery, known locally by the name 'choodai' fishery, along the coast of the Palk Bay extending from Dhanushkodi to Panakulam.

The commencement and the closing of the season might vary slightly from year to year. The fish landed during a season at Monakad near Mandapam alone usually exceeds 120,000 lb. which is equal to about 40,000 lb. of dried fish[3] fetching approximately Rs. 14,000.

The catches are composed mostly of juvenile clupeoids belonging to the *Sardinella, Hilsa* and *Anchoviella* groups. Of these *Sardinella* spp. form the major portion. The important species are *S. albella, S. gibbosa, Anchoviella* spp., *Hilsa kanagurta* and *S. fimbriata* in the order of abundance. Anchovies may not be present in all the samples and occasionally a few *Leiognathus* spp., may also be found along with the others in the catches. The fish caught during the early part of the season are on an average 30 mm. but individuals as small as 20 mm. in standard length are also fairly common and by the close of the season the average size of fish landed is 60 mm.

These young ones congregate probably for feeding on the abundant diatoms and copepods usually found in this area at this time of the year. Examination of the stomach contents shows that they feed chiefly on diatoms and copepods and occasionally on molluscan larvæ.

A sudden fall in the catch of ' choodai ' was noticed in the 1952 season. The total landings of fresh fish were only 60,350 lb. as against 120,960 lb. landed during 1951. This year (1952) the fishing started around April 9 and as usual the catches were not very large during the first two or three days. About 4,000 lb. were landed up to April 14 and on the next day 14,000 lb. were landed, this being the highest single catch for the season. Following this there was a steady decline and the fishery came to a premature close by the middle of May because the catches were extremely poor or nil. It was earlier mentioned that *Noctiluca*, although appeared on April 14, assumed a swarming proportion only on the 18th. On that day the total catch of fish was 500 lb. As already pointed out the number of *Noctiluca* was very low during May 5, 30 and June 2. On May 2, when the *Noctiluca* population was high the quality of fish caught was 525 lb. but on the 5th there was a marked increase in the fish catch (6,000 lb.) and a corresponding fall in the number of *Noctiluca* there being only 52 per c.c. Unfortunately no data are available for May 30 and June 2 as there was no fishing. From this it appears that the fish are avoiding the areas where there are dense patches of *Noctiluca*. This assumption is supported by the fact that those few fishermen who went out beyond the region of *Noctiluca* swarms located large schools of fish. Some of the local fishermen have reported that a similar phenomenon occurred many years ago in the same area but none

[3] The wet weight will be about three times dry weight as estimated in the laboratory.

of them could give the exact year in which it happened. Here is probably another instance of animal exclusion ascribable to the direct effects of external metabolites or *ectocrines*. It may be pointed out that several examples of dinoflagellate swarms accompanied by a decimation of zooplankton are found in the literature (Ref. Galtsoff, 1948 and Sproston, 1949). Fig. 1 shows the monthly average number of *Noctiluca* per c.c. and the total landings of ' choodai ' for April and May 1952.

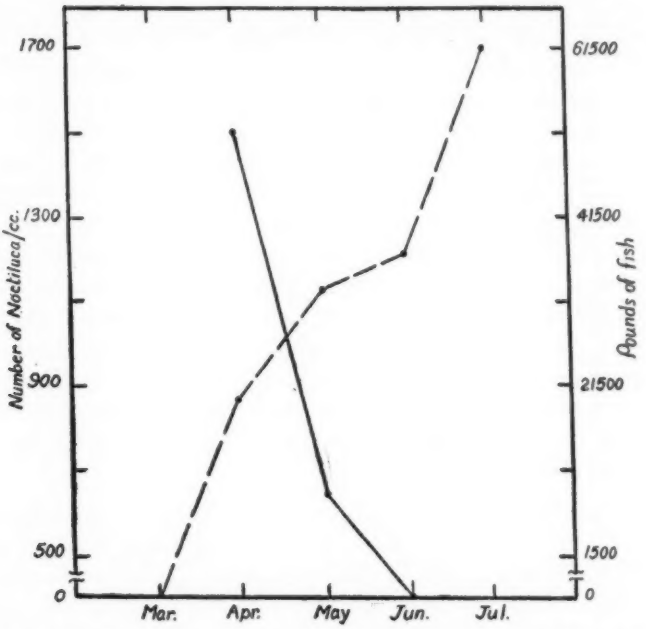

FIG. 1. Showing the average number of *Noctiluca* per c.c. (broken line) of the standardized sample and the total 'choodai' landed.

Plankton studies in relation to fisheries conducted in several places, particularly in the North Sea, have brought to light certain interesting correlations between the fisheries and the environment both animate and inanimate. As a result of these investigations on the ecological relationships there have been established what are called ' indicator species '. Some of the well-known examples of such relationships are the occurrence in certain areas of dense patches of certain species of diatoms such as *Rhizosolenia, Biddulphia* or sometimes both mixed together and the absence of herring from that region. These patches of diatoms when they occur near the main shoaling ground delay the arrival of herring or sometimes the fish are

deflected from these normal grounds. Similarly it is also well established that when in these areas there is an abundance of the copepod—*Calanus* instead of the diatoms, the herring catches are extremely good. Several other such examples of indicator species not only of fisheries but also of water movements in general are found in the literature (Ref. Bullen, 1908; Russell, 1935 *a* and *b*; Henderson, 1936; Henderson, Lucas and Fraser, 1936; Lucas and Henderson, 1936; and Corbin, 1947).

Russell (1935 *b*) has stressed the importance of watching for possible correlations between the presence or absence of certain plankton animals with that of fish and larger animals. Lucas and Henderson (1936) have attempted to study the correlation between jelly-fish and herring and they have remarked: " Detailed results suggest that the herring tend most to avoid large numbers of ' small jelly-fish' and particularly ' small *white* ' jelly-fish." The association between certain species of carangids and jelly-fish is well known and such an association found in this area has already been reported by Panikkar and Prasad (1952). Records maintained on the occurrence of jelly-fish in the inshore waters of the Palk Bay have shown that there is a regular appearance of *Dactylometra quinquecirrah* in early June. It has been observed during 1950–52 that they begin to appear in large numbers by the first week of June. The local fishermen are of opinion that this is an indication of continued good ' choodai ' fishery. This year although the jelly-fish appeared as usual the ' choodai ' fishery had by then come to a close.

From what had already been reported regarding the swarming of *Noctiluca* and its effect on fish and fisheries both along the east and west coasts of India together with the present observations and the report of the local fishermen it seems almost certain that whenever there is a *Noctiluca* swarm in the inshore waters the pelagic fishes avoid that area. It seems to be a parallel example to that of the *Rhizosolenia* or *Biddulphia* and herring relationship observed in the North Sea except that the organisms involved are different. Therefore, it is suggested that *Noctiluca* can be considered as an indicator species of a poor ' choodai ' fishery locally and in view of the remarks made by Bhimachar and George *op. cit.*, it may even be said that they indicate a poor pelagic shoaling fish fishery.

The author wishes to thank Mr. R. Jayaraman for providing the data on salinity, pH, phosphate, nitrate and oxygen saturation and Mr. K. V. Sekharan for supplying information on the total landings, the size and species composition of the fish catches.

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SYMPOSIUM ON SCIENTIFIC PROBLEMS OF THE SUGAR INDUSTRY

OPENING ADDRESS

By Dr. K. S. G. Doss, D.Sc., F.R.I.C., F.Inst.P., F.A.Sc.

(Professor of Sugar Chemistry, Indian Institute of Sugar Technology, Kanpur)

I should at the outset thank Sir C. V. Raman for having given this opportunity for sugar technologists and sugarcane specialists to meet the scientists on a common platform for exchange of their ideas. It strikes me that this meeting has profound potentialities to me in particular, who is at present in entire charge of scientific research relating to sugar industry at the Indian Institute of Sugar Technology.

Scientific problems of sugar industry are those scientific problems, which are likely to solve many of the difficulties the sugar industry is facing. There are a number of ways in which the technologist can help at the symposium. He can in the first instance straightaway point out certain problems, which would be worthwhile being tackled by the scientists. Secondly, he can put forth before this learned gathering his most important difficulties, which are bottlenecks to the full realisation of his objectives so that the scientists can conclude for themselves how best they can take part in bringing about the solution of such problems. He could also bring to the notice of the scientists any phenomena noticed by him and which are likely to be of special significance. A similar contribution can be expected from sugarcane specialists as well.

In my short talk, I am going to put general aspects of the problems of sugar industry and give a short account of the problems that strike me at present and also an account of a few attempts that I have made for solving the same.

To put in a nut-shell, the objectives in organising the sugar industry would be:—

(1) to produce more sugar;

(2) to produce cheaper sugar;

(3) to produce better sugar;

(4) to find out new avenues for profitable utilisation of sugar as a raw material; and

(5) to make use profitably the waste products of the sugar industry.

While the problems of sugar industry can be stated generally as above, the solution depends on many factors. The solution is a function of time and circumstances. It is easy to illustrate this by taking one or two of the above objectives. For instance, to produce more sugar there are many possible ways. It may be by crushing more cane or it may be by crushing more efficiently the same amount of cane. One has to look through carefully and see what exactly would help the solution with regard to the enhancing of output of sugar. It may be stated generally that the main bottleneck from this point of view at present is not in the scientific or the technological aspect. It appears to be mainly economic, as was proved by the experiment tried by the Hon'ble Shri K. M. Munshi, when he was the Minister for Food and Agriculture. Just an announcement of allowing a fraction of the sugar produced into the free market under certain conditions, transformed a production deficit to a production excess within the course of a couple of years. This shows clearly that we can have the necessary amount of raw material as well as the equipment; it will be only necessary to attract the raw material into the factory by offering all necessary inducements to the cane-grower and to the sugar factory. As I have already pointed out, this is the position at the present moment, but the solution for this problem can as well be different under a different set of circumstances.

I may as well say a few words about the production of cheaper sugar. It is wrong at the present moment to find fault with the technologist for the high price of sugar. This becomes clear from the facts that roughly 60% of the price of sugar is due to cane, about 17% Government charges by way of excise duty and cane cess, about 12% towards labour, salaries and interest leaving hardly 8% in the hands of the technologists. It is clear from this that even if we can find out a miraculous process by which the sugar from the cane is all taken out without any expenditure on materials, the cost of sugar could have been lowered hardly by one anna per seer. Thus the main difficulty in the way of cheaper sugar is the high cost of raw material. I would not go into the question as to what is the cause of this high cost. One important factor appears to be that we are dealing with small holdings and consequently, cane development work becomes an extremely difficult affair. Small holdings, it appears, have to be accepted as facts in India. We cannot wipe them out perhaps as it will mean rooting out villagers and changing their mode of life. With the advent of the community projects and extension of co-operative organisations it may be possible to minimise the evils of these small holdings.

For the purpose of this symposium it appears desirable to keep the economic and human factors in mind, but only in the background and

consider the problems facing the industry. Some of these problems arising may, when solved, act as palliatives and remove the incidental difficulties of the industry. Others may give solutions of interest from the long range point of view. The third type of problem may give results like the bolt from the blue and revolutionalise the whole industry.

Let me first consider the problems arising in the crushing of cane. One of the problems to which my attention was drawn was as to which basis is good for milling control—whether it is the *brix basis* or the *pol basis*. A careful examination of the existing literature indicated that there is a real difficulty in finding an absolutely reliable basis for judging the efficiency of milling. The milling consists in squeezing out the existing juice as efficiently as possible and bring about as good an admixture as possible of the added juice or water with the juice in the bagasse. It is the resultant effect of these two processes that we wish to judge by using the pol or the brix basis. Pol basis would have been universally adopted but for the fact that sucrose is not uniformally distributed in cane. Sucrose molecule being quite of a small size, it would be normally expected to pass through all or most of the cell walls and a uniform distribution of sucrose would normally take place if sufficient time was given. An exception to this, however, would be the portions of the juice rich in colloids which under even equilibrium conditions would contain less of sugar due to their having a high percentage of ' bound ' or ' colloid ' water. A more important cause of this non-uniformity appears to be due to the life-processes in the cells-processes whose nature and mechanism are yet baffling to the physicist and the chemist. The process of diffusion of solutes (the only mechanism by which homogenisation of the solute can take place) being an extremely slow process is easily upset by any life-process that may be occurring in the cells. What has been discussed with reference to sucrose also holds for any other solute present in the tissue fluid. It is thus seen that it is not possible to find any single constituent which would be uniformly distributed throughout the tissue fluid.

Research was, therefore, undertaken to find out as to which factor is homogeneously distributed throughout the cane. It occurred to me that osmotic pressure has the best chance of remaining constant all through the cane. Any difference in osmotic pressure would tend to bring about movement of water thereby tending to equalise the osmotic pressure. It is no doubt conceivable that such entry of water may be resisted by a counter-pressure in fully enclosed cells to some extent, but it appears that of all possible factors, this quantity has the best chance of remaining uniform all through the cane. With a view to make use of this idea for control purposes I have

suggested a new basis which I have referred to as the " isotonic pol basis". " Isotonic pol " for any juice is defined as the pol of a sugar solution having the same osmotic pressure as the juice itself. This has been examined in a preliminary way in collaboration with R. N. Agarwal[1] and results are indicative of this basis yielding successful results. The difficulty, however, is to get at an easy and reliable method for measurement of isotonic pol. This is a matter for further investigation.

It should also be pointed out that when our Atomic Energy Commission Laboratories sets up its own nuclear reactor and produces radioactive isotopes, it may indeed be possible to make use of them for carrying out perfectly reliable milling tests for judging the efficiency of mills. There are many formulæ suggested for judging the efficiency of milling. The present author has critically examined some of them and suggested the lines for further development.[34, 35, 36]

Next we come to clarification. One of our immediate difficulties is the sulphur position. About a few months back the world sulphur position in general and the sulphur position in India in particular was very critical. In fact, the allotment of sulphur for the present season to the sugar factories in this country was brought down with a view to save sulphur. The sulphur position appears to be easing somewhat now, but it is indeed possible that at any time the trouble may repeat even in a more severe form. It is, therefore, of importance to evolve methods of clarification involving either no sulphur or those using less sulphur. From this point of view it is of interest to point out an attempt that has been made at the Indian Institute of Sugar Technology. The new process worked out by Prof. Saha (Director, Indian Institute of Sugar Technology) and Shri N. S. Jain[2] in which the present writer had some part to play, consists in carrying out alkaline filtration with addition of Sindri calcium carbonate sludge, which leads to a filtrate of very high clarity. This alkaline filtrate is treated with superphosphate extract which on filtering gives rise to juice of high purity, pale colour and great clarity. Experiments are on the programme for trying this process on pilot plant and large scales.

The filter aids prepared from carbonation press mud can replace the Sindri sludge. The technique for getting the filter aid has been developed by D. G. Walawalkar and R. S. Srivastava under a scheme of research at the Indian Institute of Sugar Technology, initiated by Dr. A. Nagaraja Rao, Industrial Advisor, Government of India, and completed under the guidance of the author.[3]

It was known that the use of bauxite in clarification would help not only the removal of colour but also would help in diminishing the ash content of juices. Since bauxite is having a positively charged surface, it was surprising that it should be capable of removing cations such as Ca^{++} and K^{+}. An investigation was undertaken[4] which revealed that bauxite is incapable of taking up cations normally. For instance, no measurable adsorption was observed from calcium chloride solutions. In presence of phosphate however, there was a good adsorption of calcium ions even at a pH at which calcium phosphate is soluble. This result is of interest not only for its fundamental interest but also the important application that in cases wherein juices are deficient in phosphate, addition of superphosphate extract (or phosphoric acid) would greatly enhance the de-ashing power of bauxite.

Another idea which strikes in clarification is the development of the skimming process. Scum formation at the surface of cane juice can cause considerable degree of purification. Experiments by the present author has shown that the washed surface scums from the eliminators may contain over 60% cane wax.[5] In carrying out skimming effectively, it would be necessary to use some colloidal materials. This is being done in the Gur industry, wherein the extracts of *Deola* and *Suklai* are being used. It is to be noted however, that it is worthwhile getting these addition agents in a concentrated form absolutely ready for use. From this point of view the alginic acid being produced from seeweeds in Travancore appears to have some potentialities. This needs investigation.

Cane juice contains in it a good percentage of surface-active substances. These get removed to some extent through clarification. It is indeed of interest to find out how far these get removed. Surface-active substances affect evaporation and pan-boiling. They are also supposed to influence crystallisation. We have examined the different methods for estimating the surface-active substances by a multi-pronged attack. We worked out a new method for determining rubin numbers.[6] We tried to spread cane juice on aqueous substrates by making use of the film balance of Langmuir.[7] We tried to study cane juice[6] by the method of surface accumulation—a phenomenon discovered by the present writer[8] and independently by McBain and co-workers.[9] Finally we have been examining the influence of the surface-active substances on the electric double layer capacity of a dropping mercury electrode.[10] The last of these methods appears to have high potentialities and is under detailed investigation.

One of the lines of development in connection with clarification is the systematisation of the chromatographic technique for the purpose of

examining the extent of destruction of reducing sugars. This is of great importance from the point of view of judging the exhaustability of molasses. Hitherto we have been considering anything that reduces the Fehling's solution as reducing sugar; by adopting quantitative paper or column chromatography, it would be possible not only to characterise the nature of the reducing sugar present, but also to quantitatively determine the same. It would be a great advance if the chromatographic determinations can be quickened by any modification so that it could be adopted for routine control purposes. This is again a problem having great potentialities.

In the various stages of clarification, the control of pH is very important. The indicators are very commonly used for the purpose. The nature of the errors caused by surface-active substances on indication by indicators has been studied by the present author.[11] This is of importance in that the raw juice contains a high percentage of such surface-active substances. From this work it appears that the most reliable method for pH determination is by using the glass electrode. It is necessary however to select a pH meter based on a good electrometer valve in order to obtain reliable results with robust glass electrodes.

Several factors have been suggested for judging the efficiency of clarification. A critical study has been made of these and it is shown that the so-called clarification factor is fundamentally defective and should not be used for assessing the efficiency of clarification.[12] The purity rise is shown to be a much better index.

Regarding settling, it has been suggested that high pressure settling would be helpful, as the ' Cartesian diver ' principle would operate in bringing about quick settling. The fallacy underlying this proposal has been shown by the present author.[13]

I would like to say a few words about ion exchange. It is not the purpose to consider in detail the full aspects of the potentialities of the ion-exchange process. In general it appears that if deionisation of cane juice can be effected, the recovery of sugar would be increased and the molasses would become edible. Whereas there is a likelihood of this being adopted by a few factories in time, it is matter for consideration whether this can be adopted in our country to convert what is now the cheapest raw material for power alcohol, a material of strategic importance, into edible sugar and syrup and even if so, at what cost. It would also be necessary to make sure if there is no deleterious effect of traces of resins that get dissolved out and enter into the molasses, on the edibility.

Regarding the process of evaporation, there are two main difficulties observed (*a*) Scale formation inside the tubes, and (*b*) Rust deposition outside the tubes. Many addition agents have been described for preventing scale formation, but the results reported have been conflicting. A few new devices have recently come up under different trade names, such as the superstat, the CEPI, etc., which are claimed to prevent scale formation. The theory underlying the performance of these new gadgets is far from clear. In fact, these claims remind us of the once popular " mitogenetic effect". In a way, I am inclined to think that one method of getting rid of scale is to produce the necessary conditions for helping their precipitation in the bulk solution instead of getting deposited on walls. This may be done by introducing high speed stirrers or sonic or supersonic vibrators in a proper place in the evaporator system. This is essentially a new idea and it requires experimentation as to the optimum conditions for getting best results. It has since come to my knowledge that an ultrasonic device has been put on the market by the Swiss firm under the name " Crustex".

Many addition compounds such as calgon, have been suggested for preventing scale. The possible effect of calgon on the colour of sugar produced has been investigated.[14] It is to be pointed out however that the results obtained by these compounds on scale formation are conflicting. Further work is needed to establish the conditions under which these may be useful.

In the sulphitation of syrup, use of packed columns would greatly help in reducing the loss of sulphur dioxide. Whereas the packed columns worked out in the author's laboratory for distillation[32] would not be suitable for this purpose, the use of Raschig ring packed columns would be most convenient. A column put up by Shri Awasthy at Burhwal Sugar Factory, at the instance of the author, is giving good results.

Regarding pan-boiling, it is indeed probable that the sonic and ultrasonic radiation, is likely to be useful for graining purposes. Already some work has been reported in this connection. This requires further investigation.

It is of interest to examine how far it is economical to use active carbon on C-Sugar and B-Sugar melts, with a view to get a single high grade sugar as the product. From this point of view, work in this laboratory has shown the possibilities of manufacturing good active carbons from bagasse,[15] press-mud[16] and molasses[17]. It has been further shown that the residues after extraction of wax from press-mud can be a raw material for cheap active carbon.[18] A reliable method has also been worked out for assessing the decolouring power of active carbons.[19]

In judging as to whether the pan-boiling and crystallisation have been conducted under the most optimum conditions, it would be necessary to examine the final molasses for its viscosity at the pan-boiling temperatures as well as the degree of super-saturation, by determining the saturation temperature. The existing methods for the determination of viscosity are defective in that they often employ large volumes of molasses. This makes it difficult to obtain temperature equilibrium, which is very important for getting reliable measure of viscosity. To remedy this defect, a rolling sphere method is being developed at this Institute, taking advantage of electrical methods for finding out the rate of rolling of the sphere. Similarly, it is proposed to examine the feasibility of the Toepler's schlieren effect and optical interference techniques for designing a sensitive saturascope. From the point of view of exhaustability of molasses, it is also of interest to examine the effect of individual salts as well as reducing sugars on the solubility of sucrose as well as the crystallization kinetics. Some work has been done in the present author's laboratory.[20]

It sometimes happens that sugar crystals develop in elongated forms. This leads to difficulties in curing. The cuase of this appears to be in certain impurities inhibiting growth over certain of the faces of the growing crystal. Work in this laboratory has shown that caramel tremendously lowers the growth rate on the 100 face of the sucrose crystal.[20]

From the point of view of developing a method for controlling the size distribution of crystals in strikes, the simplification in calculation introduced by the present authors would be of interest.[21]

The final quality of sugar depends amongst other things on the extent of colour development during processing. In this connection, a start has been made on the study of kinetics of colour development in sugar solutions.[33]

In drying and cooling there appears to be scope in combining the infrared heating with vacuum so as to eliminate the deleterious effect on the quality of sugar in the usual rotary driers. In the storage and transport of sugar it is of interest to try some of the new plastic lined bags, which appear to be proof against ingress of moisture. The use of plastic film for packing confectionery has been found to be advantageous as shown by work in the laboratory.[22]

In judging the quality of sugars, the determination of ash is important. A conductometric method was worked out by Zerban and co-workers. The theory of the method has been worked out in the present author's

laboratory.[23–27] The regression formulæ connecting ash and conductivity have been derived for Indian molasses and gurs on the basis of data worked out in the author's laboratory.

A further property that has been suggested for judging the quality of sugar is based on the determination of gold numbers. This technique is very troublesome. An alternative method based on rubin numbers has been developed and applied to a few sugars.[28, 29, 30]

From the point of view of confectionery manufacture, even small traces of impurities affect the quality of sugar. This problem has been examined in detail in the author's laboratory.[31]

A scheme has been worked out for examining the quality of refined sugar cubes and data have been recorded on some samples of commercial refined sugars.[31] It has been found that electrical conductivity gives a good criterion for distinguishing a refined sugar from a white consumption sugar.

Storage of molasses is normally not a difficult problem. Occasionally, however, a very interesting phenomenon usually referred to as " Froth Fermentation " takes place completely spoiling the molasses. The phenomenon is very interesting. Almost suddenly the molasses starts frothing, rises in temperature up to 72° C. or higher, appears to boil and overflows out of the tanks. The remaining quantity of molasses gets highly caramelised and perhaps even carbonised to some extent. The cause of this extraordinary fundamental phenomenon is yet unknown. An examination of the literature is generally indicative that the first stage of the phenomenon is biochemical involving thermophilic organisms. The later stage may be a pure chemical reaction having a chain mechanism. Attempts have been made by us so far without success to reproduce this phenomenon in the laboratory by trying to initiate a chain reaction. This is a problem of great interest both from the fundamental and from the applied point of view.

Dielectric heating and capacity measurements at high frequencies have great potentialities in laboratory determination of moisture in bagasse (& molasses ?) for control purposes. This needs investigation.

The subject of by-products of sugar industry is too vast and so I have kept aloof from the subject for the purposes of this symposium.

I have given in this paper some of the ideas that have occurred to me and it would give me satisfaction if these take practical shape speedily by the co-operation of those present at the symposium and those who will read this report.

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SOME SCIENTIFIC PROBLEMS OF THE SUGAR INDUSTRY

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No other single industry can offer as many problems for solution to the numerous branches of Science and Engineering. Of immediate interest would be those, the solution of which will directly or indirectly help the Indian Sugar Industry in reducing the cost of production. The growing of sugarcane presents all the problems connected with the creation and bringing up of a eugenically perfect child, its feeding, protection against the onslaught of pests and diseases, maturity problems, etc.,—problems more complicated at each stage due to the medium of its growth, *viz.*, soil and the air and the inanimity and immobile nature. Satisfactory solutions to these problems become more difficult when they come to be examined by economic standards.

The recovery of crystal sugar from the mature cane involves several steps, each such step presenting numerous problems aimed at the perfection of the several unit processes and equipment individually and in relation to each other. The history of the development of the sugar industry in the field and in the factory all over the world represents the outstanding achievements of men of science in this field. Even so judged by the present day standards and the needs of the future we are still far away from our goal. The sugarcane, which is the most efficient fixer of solar energy of all plants, is still a highly inefficient machine, the percentage fixed by it being very small co npared to the total quantity that reaches it. The sugarcane is still a very uneconomic baby in respect of its food, water and labour requirements. The recovery of crystal sugar from the sugarcane is also a highly inefficient process involving huge amounts of power, fuel and labour, all of which should be very much smaller. The various by-products of the sugar industry both in the field and in the factory still await proper utilisation.

In so far as the cost of sugarcane forms 60% of the ex-factory price of sugar or 72% of the total cost excluding taxes, the greatest effort should be directed to increase the yield and sugar content in cane at minimum cost. The Tariff Board, 1950 reported yields of 11·7 tons cane/acre in U.P., 9·8 tons in Bihar, 32·9 tons in Bombay and 14·0 tons in the whole of India. This roughly corresponds to production of 350 lb. of sugar per acre per month in India and 750 lb./acre/month in Bombay. Compare this with

possibilities. Wilcox's studies based on plant-biological facts and ideal cultural conditions place 190 tons sugarcane per acre as the maximum. Twenty years ago the Bureau of Experiment Stations, Queensland recorded at South Johnston 144 tons sugarcane per acre and 22·9 tons of sugar per acre on a 16-month crop. In the 1950 Hawaiian Crop Ewa Plantation recorded an average of 14·05 tons/sugar per acre on the entire plantation of over 4,700 acres corresponding to 1,307 lb. sugar/acre per month. In India, Walchandnagar recorded an yield of 122·4 tons of sugarcane per acre (on 3 acres) and 14·13 tons sugar per acre corresponding to 1,900 lb./acre/month. There is thus scope for increasing the production of sugar per acre per month at least by 100%.

Varietal Problems.—While a number of varieties are showing improved results in North India, CO-419 is still the best cane in the Deccan. This variety has recently given considerable room for anxiety due to its inexplicable inferior performance in some cases. This has been ascribed to a degeneration resulting from different causes such as senile degeneration of the variety, soil exhaustion and deterioration and loss of certain essential fertility factors. Whatever future investigations may reveal, there is a very urgent necessity to intensify our breeding programme to enable the creation of eugenically and economically better varieties tailor-made to suit widely differing cane-growing regions in India.

Soils.—Systematic investigations on a uniform basis into the soils of the cane-growing regions in India both for fixing manurial schedules and for guarding the maintenance of the fertility status of the soil are urgently called for. Considering that the farmer's methods of 40 centuries are being replaced by the necessary evil of modern mechanical cultivation and intensive fertilisation most unbalanced, the importance of conserving all sources of organic matter and suitably composting and returning the same to the soil has to be realised. Methods to turn sugarcane trash and other residues rapidly and economically to compost have to be perfected. The increasing importance of maintaining a proper ratio of cattle population to cultivable land in the interest of maintenance of soil fertility and quality of crop is still to be appreciated. The " grow-more food " campaign and the pressure on land resulting from food scarcity have taken off the much needed rest and a healthy rotation. This is not safe for the soil.

Climate.—Under present state of knowledge and resources, the existing climate has to be accepted and the growing of cane adjusted to suit it. It is obvious that the Deccan and South India present more favourable conditions for sugar production and this fact is behind the proposals of shifting

the industry to the extent possible from the North, where factors other than climatic suitability were responsible for the early development of the industry.

Manuring.—The poor condition of the cane-growers in most cases is responsible for inadequate manuring of the cane crop. Such improvement as has been possible is in the factory areas, where the factories take interest in cane development. Intensive manuring is practised in most of the factory farms in the Bombay Presidency. More than 50% of the cost of cane is for manures. On the efficient use of manures, therefore, lies great scope for reducing cost of production and preventing waste of manure and spoilage of land. A proper combination of plant foods, applied according to the needs of the plant depending on age, soil and season, is the greatest need in the field of sugarcane nutrition. The following figures in respect of utilisation of nitrogenous manures reveals the need for urgent action. The nitrogen applied in lb., per ton of cane produced in different areas, is as follows:—

Punjab	3·4 lb.
U.P.	3·5 lb.
Bihar	4·0 lb.
Madras	3·7 lb.
Bombay	7·5 lb.
Walchandnagar Prize Plot	8·2 lb.
Average of prize plots in different factories	12·6 lb.
Hawaii	4·0 lb.
Java	1·6 lb.

Similar figures in respect of other manures may reveal more startling variations and wastes. The above figures indicate that while heavy manuring results in increased yields, the efficiency of utilisation of the applied manure decreases. Intensive work is called for to increase the efficiency of manure utilisation. The mechanism of nutrient uptake, needs detailed study in view of the findings that more than the concentration of nutrients it is the physico-chemical properties of the ions, at the surface of the soil colloids that determine nutrient uptake and distribution. Manuring schedules based on soil analysis, plot experiment, available plant nutrients in the soil, etc., have all been unreliable, laborious, time-consuming and what is more important, of the nature of "*Post-mortems.*" Recent studies of "Foliar diagnosis," which utilise the plant itself to analyse its nutritional requirements, excesses and deficiencies have proved of greater value. Ask the plant what it wants and at what stage of its growth and feed it accordingly.

The Clement's crop-log system which is based on the analysis of the leaves and sheaths of the sugarcane plant at different periods of growth has been a successful attempt in this direction and more than 30% of the cane grown in Hawaii are manured on this system. It is high time that the manuring schedules on the sugarcane plantations in India are placed on a more scientific basis.

Another important problem is the fixation of added nutrients by the soil especially of phosphorus which nutrient plays such an important role in plant metabolism. Placement of phosphates at the active root zone, thus increasing local concentration, beyond the fixing capacity of the soil and aerial spraying, have been so far suggested.

Another aspect which is gaining importance in recent years in the nutrition of sugarcane is the role of trace elements. Small amounts of elements like boron, copper, zinc, manganese, molybdenum, etc., appear to play key roles directly or indirectly in the nutrition of sugarcane. Promising results have already been obtained at Ravalgaon by the use of boron, zinc and copper.

Water.—Sugarcane is a water-loving plant and needs large quantities of water. Both the yield and quality of sugarcane under intensive manuring depend on the judicious control of soil moisture. The present plot-layouts and irrigation techniques, as also irrigation intervals have to be examined and revised with a view to improving the economy of water, conservation of nutrients, prevention of erosion and maintenance of vigorous growth. The water requirement of the cane crop in the Bombay-Deccan is 150" to 170" acre inches per year inclusive of rainfall and depending on the soil. Of the 140 to 150 acre inches given through irrigation in some of the lighter soils the quantity of water lost through seepage alone is between 60% to 70%. This excess water washes away the nutrients and by collecting in the lower areas spoils the land due to water-logging. Where there is enough depth of sub-soil the level of water table rises and causes salt accumulation at the surface.

Insects, Pests and Diseases—Insecticide and Weedicides.—Pyrilla, Top-Shoot borer and Stem borer are threatening the industry in many parts. In spite of D.D.T. and Gammaxene, the only successful method for controlling Top-Shoot borer and Stem borer has been the method of picking and destroying the cane infested. With Pyrilla however, Gammaxene and D.D.T. have proved useful in many parts. A recent finding by Wagle which shows promise for the control of Pyrilla is the fungus "Green Muscaradine". 2–4-Dichlorophenoxy acetic acid (Fernoxone I.C.I.) and other weedicides are

proving helpful in the control of weeds. One important aspect that needs further work is the effect of these chemical insecticides and weedicides on the soil micro-flora. Are they doing unknown harm upsetting the normal biological balance ?

Maturity and Quality.—The technique of bringing the cane to maturity by control of growth factors, timing of manurial applications and the maintenance of the plant in the state of maturity for a long period have to be perfected. The technique of controlling the irrigation towards the end of the growth period and the ultimate " drying off " as it is called has reached a high standard in Hawaii. The degree of drying off is controlled by the moisture index which at the harvest is as low as 72.

Controlling of tasselling or arrowing of cane by exposing to strong light in the night for 10 days has given 1½ tons or more sugar per acre in the experiments in Hawaii.

Fundamental investigations into the mechanism of photosynthesis, formation, translocation and accumulation of sucrose in the sugarcane plant and the controllable factors influening these mechanisms are called for. The radioactive isotopes of carbon and phosphorus are new tools available for probing deeper into these complicated processes. The Hawaiian Sugar Experiment Station has for the last two years been working with radioactive carbon dioxide and radioactive phosphorus.

Field Mechanisation.—Great scope exists in the sphere of field mechanisation in the growing of sugarcane. This aspect has already well progressed on the farms of the Bombay factories. In other places the small size of holdings and lack of financial resources are obstructions to any progress in this direction. Increasing the size of the units on a collective or co-operative basis to at least about 50 acres would help mechanisation on the lines of Australia, where one farmer with two assistants (sometimes members of his family) manages with the help of mechanised units one 50-acre block, requiring external help only at the time of harvest. Small units for preparing the land, for planting and for interculturing have been in use. Even small harvesters are under trial in Australia.

Sugar Manufacturing

Clarification.—The sulphitation process using lime and sulphur has for a long time been the cheapest and simplest method of cane juice clarification for direct consumption white sugar. The only other process in use in few factories in India is the double carbonatation process which though superior is costlier and limited by the availability of good quality

lime stone. Sulphur being an imported product and a strategic material, efforts of scientists are called for to evolve a superior and cheaper method of clarification and without using sulphur. The electrical method of clarification worked by D. N. Ghosh is a commendable attempt in this direction. The process consists in passing a D.C. through cane juice using iron electrodes and controlling the current density voltage and temperature of the electrolysis, separation of the waxy foam, precipitating the iron as iron phosphate and filtering. The report of large-scale trials made at the Experimental Sugar Factory on this process are awaited with interest. Trials conducted on a small scale at Ravalgaon showed that the method as at present involves considerable quantities of current, iron in the form of electrodes, difficulties in the filtration of the iron phosphate precipitate, etc. It is gathered that the sugar produced from this process at the Institute at Kanpur was greyish in colour and contained appreciable quantities of iron.

Treatment of midsap, *i.e.*, the syrup of about 45° Bx., from the penultimate body of the evaporator and subsequent return to the last body has been receiving increasing attention in recent years and needs to be followed up for establishing merits.

A few papers have appeared on new techniques in clarification by Prof. Saha of the Indian Institute of Sugar Technology. One of them relates to the treatment of juice at high alkalinities and high temperatures and filtration at such temperatures. The results in the laboratory have given very good rises in purity and brilliant juices. Large-scale trials at Ravalgaon have however shown that it is dangerous to adopt the process on the large scale, unless suitable equipment are designed to overcome the time factor, which is the essential requisite for success of the process. Obviously the high temperatures and alkalinities set up undesirable decomposition reactions which rapidly increase with time. Moreover this process involves more cost due to increased sulphur, labour, equipment, etc. The same objections apply to the technique of treating midsap at high temperatures and high alkalinities.

Another clarification procedure evolved by Prof. Saha is "clarification without sulphur". This process involves a preliminary treatment with Sindhri Sludge (precipitated calcium carbonate) liming to high alkalinity (10·5) filtering, treatment with phosphate and again filtering. Walawalkar has raised objections to the above claim and states that the Sindhri Sludge is highly impure for the purpose and involves very high transport costs, etc. Further that the superphosphate used requires sulphuric acid for its production, which means sulphur. These views have to be carefully examined

against the claims of Prof. Saha. A few trials of the process in the laboratory at Ravalgaon indicated likely difficulties in the filtration stages.

With the developments in Chromatography, it should now be possible to study in greater detail the variations in the composition of cane juice and the changes during the different conditions of treatment. Such a knowledge would be very useful in designing suitable clarification techniques and to understand more clearly the exhaustibility of molasses, *i.e.*, the influence of the various non-sucrose constituents in the molasses, which set a limit to the separation of sugar from it in crystal form.

Another subject of very great interest and importance is the deionisation of cane juices. Considerable progress has already been made in the manufacture of suitable resins and in the technique of deionisation. The N.C.L. is working on this. The difficulties are of an economic nature and primarily relate to the cost of regenerants. This field has large possibilities.

Process Control and Equipment.—Most of the operations at present are batch processes involving the human element, bulky equipment and non-uniformity of the results. Future trends are for continuous processing with automatic controls involving electronic and remote control devices. Such is possible with sulphitation, settling, filtration and evaporation. A considerable portion of the operation of the vacuum pans can now be automatically controlled. Continuous crystallisers and continuous centrifugals have also now been working. It will not be long before the entire sugar manufacturing is done by continuous operation with automatic electronic controls involving great reduction in labour, a major item in the manufacturing expenses. The labour per shift in an Indian factory would be of the order of 200 per shift and 500 and more including every one, as against 40 per shift and 150 for the entire factory in Queensland and Hawaii.

Steam and Fuel Problems.—The cane sugar industry is in the happy position of being potentially independent of extra fuel. Even so as against the requirement of about 25% bagasse % cane, the industry is using 35%. Though in some factories the bagasse production is much higher than 25%, the increasing value of bagasse as a raw material for various other industries, urges the utmost economy in the use of this fuel, which would mean efficient generation and use of steam. Semi or complete electrification of factories, efficient prime movers, high pressure boilers equipped with furnaces designed for efficient combustion and heat recovery are called for. Closed condensate system for boiler feed is important. Improvements in technique and equipment should also be designed to reduce the power required, when increasing quantities of by-product power become available.

The moisture % bagasse needs to be reduced from the existing level of 48%–50% to about 20% or lower and the necessary changes in furnace design made.

On the steam utilisation side, maximum economy is called for by adoption of continuous settlers and filters multiple effect evaporation with pre-evaporators and vapour heating from the 1st and 2nd bodies improved pans with mechanical circulators, cooling and reheating of massecuites and continuous centrifugals.

Chemical Control.—Considerable scope for scientific work lies in the field of chemical process control, accounting and auditing. The first essential is to introduce uniformity in the methods of sampling and analysis. Accurate, simple and rapid methods to determine sucrose in sugar house products are required to enable the more accurate sucrose basis of chemical control to be adopted. Similarly rapid and reliable methods for the determination of true solids, in sugar house materials are an urgent need. The refractometer is at present the simplest and nearest approach to the solids by drying. Methods of juice preservation also need study.

Utilisation of By-products and Wastes.—The wastes of the sugar industry include those on the farm and in the factory. On the farm the wastes are cane tops, roots and stubbles and cane trash, together forming 20% to 30% of the millable cane. The wastes from the sugar factory are bagasse (32% to 38% on cane), press cake (1·5% to 3·5% on cane) and molasses (3% to 4% on cane).

The cane tops can support a large dairy herd and farm animals. In Brazil 1·96 gallons of alcohol are recovered from cane top from one ton cane. The roots and stubbles (2% to 3%) remain in the soil and add to the organic matter.

Cane trash is being burnt and represents enormous waste. Work at Ravalgaon and Walchandnagar have shown its possible uses in making cardboard and packing material. Cane trash properly composted and returned will be of very great help to the maintenance of soil fertility.

With improvements in steam and fuel economy the bagasse can form a valuable source of cardboards, insulation boards, paper and α-cellulose.

Press cake contains valuable cane wax to the extent of 8% to 15%. This wax is being recovered at Ravalgaon on the large scale. Cane wax valued at several crores are being thrown away every year, while waxes are being imported into India. Considerable work has been done on the recovery, fractionation and bleaching of sugarcane wax. Further work is necessary

to improve the hardness of the wax and its solvent retention properties. This requires modifications in the composition of wax on the lines of the German I.G. Waxes, from Montan Wax. The N.C.L. has made some progress in this direction.

Molasses is the main by-product of the sugar industry, which awaits proper utilisation. Innumerable products can be made from molasses by fermentation, the most important so far being alcohol. From the 4 lakh tons of molasses turned out by factories in India about 24 million gallons of power alcohol can be produced. The present consumption of petrol in India is 240 million gallons per year. It is established that power alcohol can be safely mixed with advantage with petrol for use as motor fuel to the extent of 15% to 20%. It is, therefore, obvious that the entire production of molasses can be absorbed for the manufacture of power alcohol for motor fuel purposes. The recommendations of several committees and panels have so far not produced visible progress. Let us hope that the recommendations and findings at the seminar on power alcohol recently held at Lucknow under the auspicies of the E.C.A.F.E., will result in some progress in this direction.

Conclusion

Obviously the problems of the sugar industry are numerous and call for intensified efforts and team work for solution.

There are immense possibilities of such efforts resulting in increased yield and quality of sugarcane, decrease in manure, labour and manufacturing costs and in the end the overall costs of sugar production. Crores of rupees have been collected from the industry for research and development, but very little actually used for this purpose. It is in the realisation of the very great importance of intensive research and in the proper utilisation of the funds that the future of the industry rests for its survival.

SCIENTIFIC PROBLEMS OF THE SUGAR INDUSTRY

By Ram Behari Lal Mathur

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The sugarcane industry has many baffling problems to be solved and unless and until active steps are taken to overcome these problems and to solve them by actual scientific research and development, it will not be possible to place this industry on a sound economic footing. The Governments' policy of Selective Control has no doubt been able to tide over the present acute shortage of sugar so much so that our country to-day has become an exporter of this commodity. This policy as a short-term measure has brought this remarkable change and now what is required is not only to increase production but equally to produce better quality of sugar *at cheaper rates*, so as to increase the internal consumption and to compete in the foreign market. Since the price of sugarcane in India constitutes more than 60% of the price of sugar, the urgent problem before the sugar industry is to reduce the cost of production of sugarcane. This is only possible when the yield of sugar and sugarcane per acre is increased and there is profitable industrial utilisation of its by-products.

It is therefore essential that the existing research organisation be strengthened and for this Government should sanction more funds. At present very little amount is being spent on industrial, technological and agricultural research and development work. A huge research organisation is necessary to conduct large-scale experiments to evolve early and late varieties of cane so as to extend the crushing period which is at present very short as compared with other sugar-producing countries of the world and to discover disease-resisting varieties less susceptible to pests and diseases and varieties which give better yield of sugar and sugarcane per acre.

In short the main scientific problems before the sugar industry may be epitomised as under:—

1. Development of cane (*a*) to improve the yield of sugar and sugarcane per acre, (*b*) to evolve early and late ripening varieties, and (*c*) to control pests and diseases.

2. Evolving more economical and less technical methods for the manufacture of sugar.

3. Improving the technical efficiency of working or minimising manufacturing losses.

4. Industrial utilisation of its by-products.

I shall now deal with some of the points in greater details.

1. Cane Development Work

One of the chief handicaps for the present state of sugar industry in India has been the low yield of sugarcane per acre, which is for all India about 15 tons per acre as compared with 50–60 tons per acre in Hawaii, Java and other progressive sugar-producing countries of the world. The sugar yield per acre for the whole of India is also very low, about 1·5 to 2·0 tons per acre as compared with 8·0 tons in Hawaii and Java; 5·07 tons in Brazil; 3·65 tons in Japan and Formosa; and 3·06 tons in Australia. The higher cost of production of sugar is mainly due to higher cost of the raw material, which is poor both in yield and quality. Unless the yield of sugarcane increases to 30 tons per acre and sugar recovery to 12% on cane, this industry cannot compete in foreign market under any circumstances, so much so that it cannot even survive without protection.

At present the quality of sugarcane is not much of a problem as due to the comprehensive research work done in this direction at the Sugarcane Breeding Station, Coimbatore, and its sub-stations, several standard varieties of sugarcane have been discovered which give greater yield and more sugar. The improved CO varieties now occupy about 90% of the cane area in this country as against that of 11·6% in the year 1928–29 and 75% in the year 1935–36. The achievement of Dr. C. A. Barber, Sir T. S. Venkatraman and Dr. N. L. Dutt and others of this Station in the evolution of improved varieties of cane will long be remembered in the history of sugarcane breeding and development in India and abroad. The varieties evolved there have not only significantly improved the agricultural economy of India, but some of them have been lately introduced and planted in foreign countries like Australia, South Africa, South America and others.

It is regrettable to note that with all the improved "CO" varieties of cane there has been no significant improvement in the yield of sugarcane per acre, mainly due to lack of adequate scientific approach to cane agriculture. It should be clearly understood that the fall in yield not only affects the cane supply to sugar factories, but also affects fortunes of more than ten millions of people who depend on cane agriculture. If the work of cane agriculture and development is carried out carefully on scientific lines, the target figure of 900 maunds of cane per acre in U.P., Bengal and

the Punjab; 700 maunds in Bihar and 1,200 maunds in Bombay and Madras on the average will easily be achieved in the next few years. This sorry state of affairs may be due to the fact that the improved varieties of cane as released by the Research Breeding Stations very often do not give the same results as expected of them under varying climatic conditions and nature of soil. It is therefore essential that the study of the special character of the soil for each factory should be made *as the same cane may give quite different yields of cane per acre on different soils and also on the same soil in different seasons.* Since the work of this nature has not been done so far at the door of the cultivators, no satisfactory improvement in cane agriculture has been achieved. In the interest of cane agriculture it is suggested that a farm of at least 1,000 acres should be attached with each factory for use as centre of agricultural improvements and as seed nurseries. Soil scientists and sugarcane plant physiologists of the Government as well as the factory technical personnel should work in close association in these farms and study whether the improved varieties of cane as released by Sugarcane Breeding Research Stations can with advantage be grown in the factory zone as well. A work of this nature in the factory zone will give far improved results in a much shorter time.

(*a*) *Irrigation and Manuring.*—Substances that act as a growth factor are fertilizers, water and gases like carbon dioxide and oxygen of the air and sunlight. All these factors are indispensible. If there is a shortage of any of them the growth is stunted.

Successful agriculture depends on the joint action of the abovementioned factors, all of them indispensible. Water is more indispensible than the others. Experience has shown that even the best variety of cane does not give the results as expected of it due to lack of timely irrigation facilities in cane areas coupled with the well-balanced organic manures and suitable fertilizers.

Soil scientists and plant physiologists should therefore labour to determine the amount of different fertilizers needed to produce the maximum yield of cane. There should be enough of the three principal elements Nitrogen (N), Phosphoric acid (P_2O_5) and Potash (K_2O) for a maximum yield. The farmer should know how much of these elements are already present in his soil and how much new fertilizers must be added. The physiologist should actually determine the water requirement of the sugarcane in the factory zone in relation to the fertilizers added to the soil.

A pressing need in sugarcane as well as in all agriculture is for a procedure by which both big and small farmers can easily and inexpensively

make enough field tests to disclose the fertility status of all their cultivated lands. The Mitscherlich's table of one plot test to determine the Law of Diminishing Return will be very much useful.

The sources of organic manure in the world and in this country are limited and this has led to the manufacture of inorganic fertilizers. It is hoped that the Sindri Fertilizer Factory will soon solve this big problem. For organic manures the State should formulate and popularise plans for the utilisation of compost and farmyard manure and other organic village manurial sources. The efforts in this direction require greater publicity. Even now we are not utilising the night soil and other refuse for composting which costs very little but which would give additional revenue. It is hoped that the States would make suitable arrangement for composting this in villages if possible, municipalities and towns and would give technical aid and demonstrations where required.

(*b*) *Control of Pests and Diseases.*—One of India's most serious problems in recent years has been the damage done by sugarcane pests and diseases to the extent of many lakhs of rupees annually. In the beginning the diseases were mostly confined to " Desi " or indigenous varieties of cane in Northern India. But at present it is gradually spreading to several "CO" varieties as well. As a result of scientific tests made so far, a few practical methods have been evolved for protection of cane against Red Rot, Smut, and Mosaic diseases and for controlling borers and other insects. But these methods have not been amply demonstrated so far to the cultivators. An intense research in the direction of curative methods is urgently needed.

(*c*) *Small Holdings and Their Disadvantages.*—The Indian Agriculture is also backward and cannot progress fast due to small and scattered holdings, which are proving uneconomical to the farmer. He does not know where to go for money to effect improvements or even to meet his ordinary expenses for cultivation. The consolidation of small holdings and co-operative farming will give them the desired salvation and improvement. It should also be remembered that there is a shortage of agricultural labour and the Indian cattle is almost useless and uneconomic in agricultural operation. Hence there is a great necessity for *partial* mechanisation in cultivation which, though difficult for an individual cultivator to adopt, may become easy by promoting co-operative farming. Our cultivators unfortunately are not co-operative minded and the States will have to make strenuous efforts to educate them.

Cultivators in the East U.P. and North Bihar have very small holdings, while those in the West have comparatively bigger ones. In small holdings

there is no rotation of crop and the cane is grown year after year as it fetches better price and the land consequently becomes cane sick. This also gives low yield and poor quality of cane followed by diseases. Consolidation of holdings will give a fair solution.

2. EVOLVING MORE ECONOMICAL AND LESS TECHNICAL METHODS FOR THE MANUFACTURE OF SUGAR

Simultaneously with the efforts in the direction of improvements in cane cultivation, technological researches on improved methods for the manufacture of cheaper and better quality of sugar should be evolved so that the cost of production of sugar may be further reduced. So far very little work has been done in this direction and a more intensive research is needed. Professor J. M. Saha, Director of the Indian Institute of Sugar Technology, has evolved a process " White Sugar Without Sulphur ", but his experiment so far is based on laboratory scale only. It is difficult to say at this stage how far his new process is practically workable and economically sound on the industrial scale. But still his efforts in this direction deserve appreciation as it might solve the problem of the shortage of sulphur which is largely imported. Professor D. N. Ghosh of the Science College, Patna, has invented and patented a process for the clarification of cane juice by an electrical process without the aid of chemicals and of heat. The process seems to be theoretically correct and economically sound but it has not been tried on commercial scale to my knowledge, and no definite opinion can be expressed at this stage. In these days of limited profits to sugar industry, sugar factory owners do not easily come forward to undertake the risk of a trial with a new research in their factories. It is therefore suggested that the Experimental Sugar Factory attached to the Sugar Institute, Kanpur, should be enlarged in capacity and equipped so well as to enable the practical technologists and the research scholars to experiment there on the industrial scale. The humble writer has some of his own valuable researches waiting for such trials.

3. IMPROVING THE TECHNICAL EFFICIENCY OF WORKING

Regarding the technical efficiency, the industry has achieved a fairly high efficiency in working as compared with the other leading sugar-producing countries of the world. The over-all efficiency (which denotes the technical efficiency of the factory) has gone up from 83·5% in 1934 to about 86% now, which is better than Natal (82–83%) and is equal to Mauritius, Java and Cuba, but is still less than Hawaii (89%), and Formosa (91%). The recovery of sugar has also much improved in recent years.

There is still scope for improving the efficiency of the factory. The existing plant must be designed to avoid the basic causes of lack of efficiency. The most skilled operator regardless of his ability and devotion to work cannot adjust and balance the operation as effectively as can automatic instruments. The utilisation of steam flow, pressure and vacuum recorders attached to various units not only results in *a more uniform operation but also in saving much fuel.* It must be clearly understood that *the secret of obtaining higher over-all efficiency depends on the regularity in its operational conditions, regularity in the juice and steam flow and maintaining high vacuum without fluctuation.* Unless these conditions are improved to the optimum with the help of the automatic instruments and devices, all considerations on the improvements in the construction and design of the plant will prove worthless.

I sincerely feel and regret to note that the importance of technical and scientific control has not been realised fully so far by most of the factory owners.

Persistent demands for increasing labour wages also forces us to replace hand labour with mechanical and automatic operations.

4. Industrial Utilisation of the By-Products of the Industry

The cost of production of sugar in a very large measure would also depend on the profitable utilisation of the two chief by-products of the industry—molasses and bagasse.

(*a*) *Bagasse.*—It is an important by-product of the industry and not a waste product. It is used as fuel in sugar factories, where power and steam are important cost items in the manufacturing process.

Our country is short of cellulosic materials and bagasse can well be utilised as a source of cellulose in several essential industries, as for example Paper, where foreign currency is involved. Bagasse on dry weight contains about 18% lignin, 15% water-soluble substances, 45% cellulose and the remainder hemi-cellulose. The prospects of its utilisation in any other industry, however, depends upon the economic supply of the alternative fuel. Fortunately our country is in a position to replace bagasse by coal and the bagasse so saved can be used with advantage in other industries.

Our cane varieties in general have a large percentage of fibre, 15% and above. The Java cane has about 11–12% fibre and factories operating there under conditions of improved heat balance do not require extra fuel. Potentially our factories have surplus bagasse. With similar improved heat balance the saving in term of fibre in bagasse should therefore be about

3 to 4% on cane. A further surplus of fibre by about 2% can be achieved by making the plant semi-electric. The total surplus in that case will come to 5 to 6% of fibre on cane. Bagasse produced by our mills is in the neighbourhood of 5 million tons every year, almost the whole of which is used as fuel. From the above calculations about 2 million tons of bagasse can therefore be economically used for other important industries in India. In U.P. and Bihar where more than 75% of sugar is made the surplus bagasse will be about 1·5 million tons, and the remaining 2·5 million tons of bagasse will be used as fuel. If coal replaces one million tons of bagasse, then the total bagasse available for other industries will be to the extent of 2·5 million tons. In U.P. and Bihar sugar mills are practically clustered which offers a definite advantage in the transportation of bagasse from several mills in the neighbourhood to work a central paper mill or a chemical industry.

Cardboard manufacture from bagasse has been undertaken by some enterprising industrialists but paper pulp manufacture entirely from bagasse has yet to be undertaken. Availability of bamboos and Sahai grass for the production of papers is limited, because the former is used for a large variety of purposes and the latter grows in particular areas.

The other outstanding successful industrial utilisation of bagasse as a raw material has been in the manufacture of wide varieties of insulating and hard board products for the building industries and for providing insulation against heat, cold and sound. Louisiana, Hawaii, Australia, Cuba and England are doing so. The Japanese are said to have manufactured insulating building board from Bagasse in Formosa. According to Dr. C. E. Lathrop, " bagasse, because of its long tough, springy character is a superior raw material for the manufacture of insulating building board products and that it is not possible by known means to produce a fibre of similar properties from wood." Intensive experiments conducted in America have shown that fibres of the rind and bundles are excellent for producing writing and fine papers.

Bagasse is not only a source of useful fibre but also is an excellent source of chemicals. These chemicals are formaldehyde, furfuraldehyde and various ketones.

As bagasse contains a large proportion of pentosans, it is a very suitable raw material for the production of furfural which has a wide range of uses, commencing from the synthetic manufacture of Rubber and Nylon to the separation of vegetable oils into food and paint oil constituents.

(*b*) *Molasses.*—Production of molasses by central sugar factories is of the order of 4 to 5 lakh tons annually and the disposal of the same was a problem of great headache to the industry even a few years back. But use of alcohol in admixture with petrol due to Government policy has now relieved the situation. Price of molasses fixed for this purpose, however, continues to be unremunerative, as price of alcohol is linked up with that of petrol. Molasses contains about 28 to 30% sucrose, 16 to 20% reducing sugars and a substantial quantity of aconitic acid, besides valuable nitrogenous substances, potassium salts and phosphates.

Molasses is now sold to the distilleries of Northern India at a price less than Rs. 6 per ton ex-factory, whereas the actual sugar content of a ton of molasses has a value of Rs. 175 to Rs. 200 at the present cost price of sugar. If the price of sugar is to be reduced it is not sound economics to remain satisfied with this price of molasses which may go down still lower if the petrol becomes cheaper. It has not been possible so far to extract sugar of molasses profitably but with a more intensive scientific research it should be possible to utilise the different constituents of molasses in producing other useful substances. Bio-chemical methods may come to our aid in producing lactic and citric acids. How to improve yields of acetone and butanol requires our concentrated attention.

Molasses contains an appreciable quantity of aconitic acid. If in near future, industries requiring aconitic acid and its derivatives are developed, molasses will be a very good source of the same. Our efforts should be directed to develop a suitable method of recovering this particular acid.

There are still many more possibilities to be explored and the field is still a fertile one in which important developments may confidently be expected in the future.

Conclusion

The sugar industry has many baffling problems to be solved. The great problem before the industry is to produce sugar at cheaper rates, so as to increase the internal consumption and to compete in the foreign market, when Protection is lifted. This object will only be achieved if the cost of production of sugarcane is reduced by scientific methods of cultivation and processes evolved for the profitable utilisation of its by-products. A huge research organisation is necessary to conduct large-scale experiments in the domain of agriculture and technological developments. Early and late varieties of cane should be evolved to extend the period of crushing, especially in U.P. and Bihar. How to produce disease-resisting varieties and the type of cane which gives better yield of sugar and sugarcane per acre, needs urgent

research. Less technical and improved methods for the manufacture of cheaper sugar should be discovered by intensive technological researches. Pests and diseases play a great havoc with sugarcane crop. Although some preventive methods have been evolved which need greater demonstration, curative methods are yet to be investigated. Farmer should know the condition of his soil and what fertiliser is needed. A pressing need in sugarcane agriculture is for a procedure by which both big and petty farmers can easily and inexpensively make enough field tests to disclose the fertility status of the soil. The Mitscherlich table, of one plot test to determine the law of diminishing return will be very useful. Our country is short of cellulose materials and bagasse can well be utilised as a source of cellulose in several essential industries like paper and insulating materials. Availability of bamboos and Sahai grass for the production of paper is limited and therefore bagasse can be used in its place. Coal can be used in place of bagasse to develop subsidiary industries. The use of molasses for the production of power alcohol has now removed the great headache for its disposal, but the price of molasses fixed for this purpose is unremunerative for the industry, and it is suggested that a part of it may be utilised to produce chemicals like aconitic acid, lactic acid and citric acid.

Government should allot more funds for the development of the by-product industries.

My grateful thanks are due to Shri Rajindar Lalji, the Managing Proprietor of the U. D. Sugar Mills Co., Ltd., Shamli, for his encouraging me to write this article and to attend this Conference. My sincere thanks are also due to Shri N. C. Dutta, my senior assistant, for assisting me in this paper.

THE ELECTRICAL COAGULATION OF CANE-JUICE COLLOIDS AND A NEW SUGAR TECHNOLOGY

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In the present communication it has been shown how the problem of cane juice clarification can be best attacked by a novel electrical method, based on sound Laws of Physical Chemistry, and briefly summarised as follows:— Raw cane juice contains mostly water in which is present in dissolved state the sucrose, some gums and vegetable proteins, while dirt, fibre hemicelluloses, etc., are found in the suspended state. The dissolved non-sugars in the juice render it unfilterable and form the main problem in its clarification. In the existing method of clarification excess of lime is added to the normally acidic juice to render it alkaline and then carbon dioxide in the carbonation process and sulphur dioxide in the sulphitation process are blown into it till neutral. The solid calcium carbonate and calcium sulphite particles formed as a result of this reaction remove the non-sugars by adsorption more or less completely depending on the quantities of solids formed and more correctly the total juice-solid interfacial area created in the body of the juice for the adsorption process to take place. It is well known that the addition of lime to raw cane juice is the root cause of most of the troubles in sugar factories such as heavy molasses formation, scale formation, etc., and it was decided to avoid its addition altogether and by taking advantage of the adsorption of non-sugar at the gas-juice interfacial area by generating electrolytic gas bubbles when a thin column of raw cane juice, enclosed between two iron plate electrodes, is subjected to direct current electrolysis under low voltage. It is a well-known principle of Physical Chemistry known as the Theory of Peptisation or Protection of Colloids according to which in a mixture of colloids, the lyophylic or dissolved colloids often exercise a profound protective influence on the lyophobic or suspended colloids. Raw cane juice is such a mixture of colloids, the dissolved colloids being gums and vegetable proteins, which protect the suspended colloids, *e.g.*, dirt, fibre, hemicelluloses, etc. As a result of almost complete removal of the dissolved colloids mostly as froth by adsorption at the electrolytic gas-juice interface, as outlined above, the suspended colloids automatically settle and the juice becomes readily filterable. With the removal of the acidic dissolved colloids and the electrolytic action on the dissolved alkali

salts, the acidic juice soon becomes alkaline and the trace of iron that finally leaks into the clear alkaline filtrate is removed by treatment with superphosphate.

The use of lime and sulphur has thus been altogether eliminated in this new sugar technology and the process consists of exposing raw juice heated to 80° C. to the action of direct current in a battery fitted with iron plate electrodes, when the stabilising lyophilic colloids which are negatively charged, are almost completely removed, partly by direct contact with anode plates, partly by charge neutralisation with the positively charged trivalent iron ions generated by the current and mostly by adsorption at the electrolytic gas-juice interface as froth. The removal of the dissolved lyophilic colloids results in the automatic coagulation of the suspended lyophobic colloids and the Ph of the juice rises from 5·4 to 7·2. This electrically exposed juice readily filters and the faintly alkaline filtrate (Ph = 7·2) is rendered faintly acidic (Ph = 6·8) with the addition of superphosphate and heated to boiling. This on filtration gives the finally clarified, sparkling and almost water clear juice which is sent to evaporators.

The electric process ran successfully at the Kanpur Experimental Factory from 28–12–1951 to 11–1–1952 in the course of which about 500 mds. of white sugar crystals were produced for the first time with electricity as the clarifying agent, thus confirming the theoretical expectations as earlier outlined.

Some interesting results achieved during this trial are as follows:—

(i) " Properly treated juices filtered well."

(ii) " Ph of the first filtrate was found to be 6·7 to 6·9 (expected 6·9 to 7·2) and that of the second filtrate to be 6·4 to 6·6" (expected 6·6 to 6·8).

(iii) " *Clarity*:—The clear juice obtained by the electrical process was brilliant and light in colour and on concentration to even 75 Brix did not develop any turbidity."

(iv) " The juice and syrup obtained by this process were very clear."

(v) " *Purity rise*:—The maximum rise obtained was 3·51 in terms of sucrose purity." The expected rise is 4 units by the electrical process as against less than 1 unit by the D.S. process and 3 units by the D.C. process.

(vi) " Mixed juice reducing sugar %—0·557, clear juice reducing sugar %—0·600 " (expected small change if any).

(vii) " *CaO content* :—No lime being used the CaO content in the clear juice was low, *viz.*, 90·5 mgm. litre as against 500 mgm. litre in the usual sulphitation process. This fact is bound to reduce scaling in the evaporator and prolong the duration of its working. Such was found to be the case on the inspection of the evaporator after about 14 days working."

The Electrical process thus confers a blessing hitherto unknown in the sugar factories of the world. With its operational simplicity and quickness, the Electrical process, without using lime and sulphur, claims a better clarification of raw cane juice than by the existing Double sulphitation and Double carbonatation processes, and the above proves beyond doubt that it has fully established its claim during its maiden run at the Kanpur Experimental Sugar Factory.

According to calculations given in the earlier communication on the subject, the ratio of the cost of clarification by the three methods is approximately as follows:—

Double sulphitation; Electrical; Double carbonatation = 1 : 1·7 : 2·9 with corresponding rise in clear juice purity in the ratio 1 : 4 : 3. It was desired to verify these figures during this trial and although the data on purity rise were observed to be fairly satisfactory, unfortunately, the data collected during this short trial for calculating the cost of electrical clarification were found to be incorrect owing to some heavy leakage of electricity through the juice delivery drains of the batteries, which was detected and stopped only on the last day of the run. It is expected that in the next run sufficient data in this direction will be collected which will not be too widely divergent from the figures given above.

Apart from eliminating the use of sulphur and lime in the sugar industry, some other novel features of this invention are as follows:—

(i) Higher recovery of sugar owing to higher purity rise of the clear juice.

(ii) Less formation of molasses.

(iii) Relatively more edible nature of the molasses as it contains no lime and sulphur compounds.

(iv) Lower mud % cane, as no solids in the shape of calcium salts are being externally added.

(v) Complete and cheaper recovery of the valuable cane wax, the solvent extraction of which from the existing processes is unprofitable.

(vi) The simplicity of operations in the electric process automatically eliminates the liming, the sulphitation and settling stations, causing considerable saving in labour factory equipments and floor area.

(vii) In the absence of lime salts in the clarified juice evaporator troubles due to scale formation are also avoided as has already been confirmed during this trial.

THE BREEDING OF SUGARCANE

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I. Introduction

The main scientific problems which fall within the purview of the Sugarcane Breeding Institute, Coimbatore, are those connected with the breeding of improved varieties and thus relate chiefly to the raw material of the Sugar Industry, *viz.*, the sugarcane supplied to the factories. The sugar industry in India stands on a somewhat different footing as compared to other industries, as the bulk of the cost of the finished product, *viz.*, sugar, is represented by the price paid for the raw material which is nearly $\frac{2}{3}$ of the cost of production of cane sugar. As such, the protection which was granted to the sugar industry was one of protecting a particular branch of agriculture until such time as improvements in methods of cultivation and developments in research enable the agriculturist to increase his yield per acre while maintaining or increasing his profits.

The crux of the problem is, therefore, raw material, not merely because of the high proportion of its cost in the total cost of cane sugar, but principally because the quality and the output or yield per acre of the raw material were very poor, indeed, the poorest in the world. The yield of cane in the sub-tropical North India (which represents 80% of the total acreage under cane in India) was only 10 tons per acre against 70 tons in Hawaii, 60 tons in Java and 30 tons in Cuba. This yield has, with the spread of improved varieties of cane bred at Coimbatore, risen by 50%, but there is still a long road to travel if we are to compete on equal terms with other sugar producing countries and place our sugar industry on a sound basis. It is true that in Cane Competitions yields of over 60 tons per acre have been recorded in Uttar Pradesh and elsewhere in North India and over 100 tons per acre in the Deccan Canal Area of the Bombay State, but what is to be aimed at is high or at least fairly satisfactory average yield of cane over the main cane-growing areas. The potentiality of high yields of Co. canes in cultivation has thus been demonstrated by the high yields in Cane Competitions and the aim of satisfactory yield by the average cane-grower can be achieved by proper cane development. Cane Development Departments have now been set up in the chief sugarcane growing States and the Indian Central Sugarcane Committee has also sanctioned long term Cane Development Schemes, and progress in this behalf may now be hoped for.

In the present note only the technical or scientific problems are briefly touched upon. These problems are varied and of a complicated nature.

II. CANE-BREEDING PROBLEMS

The very first problem which the Institute had to tackle was whether the varieties that had to be crossed would flower under Coimbatore conditions and set seed satisfactorily. So far as the technique of crossing and raising of seedlings is concerned a satisfactory technique was evolved and is in vogue for the last four decades with slight modifications depending upon the combinations that have to be effected each year but the problem of flowering still remains. Quite a few varieties have not yet become available for hybridisation because they have not flowered so far while others are unavailable for effecting certain desirable combinations as the disparity in the time of flowering is such that they cannot be mated. A scheme on Sugarcane Physiology has now been in existence for the past few years devoted mainly to the study of the physiology of flowering in sugarcane and some details of the progress made in this direction are given in a later paragraph.

The second problem was whether the canes bred at Coimbatore will suit the rather rigorous climatic conditions of the sub-tropical North Indian regions where the bulk of the area is situated. To ensure the success of Co. canes in North India, Dr. Barber and Dr. Venkatraman took the unusual step of utilising the wild *S. spontaneum* in breeding and this paid rich dividends as the very first seedling of a direct cross between *S. officinarum* and *S. spontaneum*, *viz.*, Co. 205, was an immediate success in the Punjab. Only the Coimbatore form of *Saccharum spontaneum* and later Glagah, the Java form have been used at Coimbatore. A few seedlings have been raised from Burma spontaneum also. But there is such a wealth of *S. spontaneum* forms in India that this Institute has now organised an exploration for collecting the wild forms of *Saccharum* and allied genera from all parts of the Indian Union in its Spontaneum Expedition Scheme. An account of this important work is given in a subsequent section.

Another problem of some importance which presented itself was that no very satisfactory method was available for assessing the male viability. Attempts at germination of sugarcane pollen *in vitro* had failed and the storage of pollen in a viable condition was attended with its own difficulties. Ultimately a method for successfully culturing sugarcane pollen was evolved, but the storage of pollen is conditioned by the fact that howsoever well stored, like certain other graminaceous pollen, it does not retain its fertilizing capacity beyond 48 hours. On the pistil parent side, tests on the stigma receptivity were made and the time taken in fertilization determined.

The problem of evolving canes suitable for local areas in the various States is a perennial problem because for one reason or another varieties go out of cultivation; moreover better varieties are bred and come to replace the older ones from time to time. The breeding of better and yet better varieties is thus a continuous process. In the Punjab tract in North India Co. 205 gave place to Co. 285. The cane which proved to be almost an universal cane for the sub-tropical belt was Co. 213 and was in cultivation from East Punjab to West Bengal, and formed the backbone of the sugar industry for a number of years. It, however, fell a prey to red rot disease and had to be replaced by other varieties. Co. 312 and Co. 313 then came into the picture, but Co. 312 has also shown susceptibility to red rot and needs replacing. There has been during relatively recent years a reorientation in the type of canes for North India. It was felt that slightly thicker or medium thick canes could with advantage be cultivated in North India provided they could withstand the extremes of temperature. The advantage would be that with better cultivation and irrigation they could give much higher yields which would have been impossible with the thinner reed like older varieties. Special combinations were therefore devised and in due course canes like Co. 421 and Co. 453 which are somewhat on the thicker side as compared to Co. 213 and Co. 285 have come to occupy fairly extensive areas in North India. This process is being continued and the search for new parents from among the Co. canes themselves most of which are tri-species hybrids continues each year as more experience is gained.

The breeding of suitable varieties for the tropical States of Bombay and Madras was taken up at a relatively later stage as compared with the canes for North India and fortunately in Co. 419 an almost universal cane for the tropical conditions in Bombay, Madras, Hyderabad, Mysore and Orissa was evolved and shows very good growth and yield. Other recent canes are Co. 449 and Co. 467. Naturally the parents employed for breeding canes for peninsular India are very different from those employed for breeding canes for North India as in the canes for the South a preponderance of *S. officinarum* can with advantage be woven into the parentage. Care, however, is taken that a certain amount of hardiness is also introduced.

The interesting work on the intergeneric hybrids including the sugarcane × bamboo hybrids is now reserved more for academic studies in the Cyto-genetical Section excepting crosses with *Sorghum* for evolving early canes and those with *Sclerostachya* and *Narenga* for disease resistance.

Breeding for earliness and high sugar is an aspect which concerns the sugar factory most. High sucrose and earliness in ripening have been found

difficult to combine with satisfactory yield. However, Co. 313 among the earlier canes and Co. 527 among the relatively later series have fulfilled the requirements to some extent. Among the recent canes Co. 630, Co. 644, Co. 659 and Co. 686 combine in themselves early maturity and fairly high tonnage, but these are as yet in the experimental stage. In connexion with the selection of early ripening varieties studies on the anatomy of cane leaves have indicated correlation between cell size of leaf and maturity. The size of the stomata in both lower and upper epidermes and the bulliform cells in the upper epidermis have been noticed to be smaller in the early ripening varieties as compared to the late maturing canes.

Recently the formulation of breeding aims for production of varieties with certain specialised characters has become more necessary with the continually changing outlook both from the agricultural and factory points of view. Attention is now being paid to the production of (1) canes which do not have pith and are not hollow, (2) chewing varieties, (3) disease-resistant varieties, and (4) varieties with good manufacturing qualities. After an elaborate study of the varieties combinations have been devised which produce canes that show no tendency to pith formation or hollowness in the centre. The pithy portion does not contain juice and pithy stalks are less in weight. One other drawback with stalks having pith is that the canes tend to dry up quicker after harvest as also in the field when kept on in hot weather. There has been a demand for chewing canes both in urban and rural areas. This in some respects has been difficult of achievement for chewing qualities and the yield particularly under North Indian conditions are hard to combine. Canes like Co. 798, however, have been recently released for trial and may succeed at least in some tracts.

Attention is also being devoted to the production of varieties resistant to red rot. This aspect deserves serious attention since some of the commercial varieties are falling a prey to this disease. In the work on breeding of disease-resistant varieties with particular reference to red rot, crosses between highly susceptible varieties and those which are moderately susceptible have shown a great deal of variability in the matter of disease resistance and the data available indicate the need for caution. It is now felt that the best method of evolving red rot resistant canes would be to inoculate the seedlings and study them for red rot resistance and release only those which are found to resist the disease.

As regards the manufacturing qualities, a beginning has been made in the study of the chemical composition of the juice as it affects the quality of (1) gur as manufactured by the average cane grower and (2) white sugar

in factories. The prominent Co. canes in cultivation have been analysed for total nitrogen, protein nitrogen, ash and colloids. The various constituents of the ash, *viz.*, lime, phosphoric acid, sulphate and iron were also determined. It is proposed to continue this study to seedlings of known parents to see how far they contribute good and bad juice characters to their progenies.

III. Cyto-genetical Problems

A Cyto-genetical Section is an essential limb of any plant-breeding project. Studies on cyto-genetics of sugarcane have been conducted at Coimbatore by Drs. Janaki Ammal and N. Parthasarathy and now by Dr. Raghavan. The studies have revealed that cyto-genetically even the simplest of sugarcane material is highly polyploid with an equally high degree of genetical impurity. To this extent in sugarcane breeding there must always be a very large element of chance.

The main problem is to bring sugarcane breeding in a line with other crops which are within the operation of mendelian heredity. This can be achieved by simplification which in its turn may be expected to re-establish a fairly high degree of purity. One way of achieving this is through haploid parthenogenesis of which no sign exists so far. Another is by continued back crossing with forms which are likely to be the progenitors of sugarcane. This is also being done. But the problem is to bring about an elimination of all chromosomes other than those of officinarum basic complement. Study of the meiotic configuration in such back-crossed progeny has thrown light on the constitution of *S. officinarum*. There has also been found recently chromosome eliminations *en bloc* and this makes it possible for different chromosomal races to come into existence and it is suspected that this phenomenon also plays a very important part in sugarcane breeding. Cytoplasmic inheritance has also been noticed and it is presumed that in sugarcane breeding more importance has to be paid to the mother than to the staminate parent.

IV. Spontaneum Expedition Scheme

Shri R. R. Panje is in charge of the Spontaneum Expedition Scheme. The strenuous work of the actual exploration is now drawing to a close and the study of the botanical and agronomical characteristics of the over 200 forms as also of the exotic types imported from South-East Asia, the Middle-East and the African areas has been taken up.

On a very close study of the characters depends the utilisation of these forms in breeding work. The special value of the wild sugarcane lies in

the fact that apart from at least two sub-species, *Saccharum spontaneum* has several ecological races which are adapted to a variety of habitats, and these ecotypes are to some extent associated with a fairly wide range of chromosome numbers. Among the forms collected are giant types, thicker and broader-leaved than some of the cultivated canes; dwarf, bushy types; spreading types; rhizomic forms and types with certain unusual morphological characters. Observations on the distribution and the ecological adaptations of these forms in the original habitats against the behaviour of the trans-plants have given indications of the extent of utility of the forms in breeding. Selections of genetic stocks have been made from out of a wide range of useful characters, *viz.*, habit, growth-vigour, drought-resistance disease-resistance, tolerance to salt, sugar content, etc. Some of the selected forms are being tested for the heritability of the desirable characters. Work on vegetative and floral morphology, starch content, epidermal patterns and chromosome numbers has shown certain interesting trends within the species, though the complete picture will take yet some time to emerge.

The taxonomy of *Saccharum* and the allied genera needs a close scrutiny. The delimination of this genus from the allied genera has undergone changes and the question of the validity of certain genera is awaiting further research. A beginning towards this has been now made through studies on the morphology, anatomy, ecology and distribution, as also on the cytology and biochemistry of the forms and species within *Saccharum*. These aspects together with the botanical and agro-physiological assessment, the pre-testing of the genetic stocks and the building up of presumptive parents out of the wild material constitute by themselves a whole branch of research bearing on sugarcane breeding with a far-reaching applicable value.

V. SUGARCANE PHYSIOLOGY SCHEME

Studies on the physiology of flowering in sugarcane have been conducted at Coimbatore by Mr. N. D. Yusuf and now by the present Sugarcane Physiologist, Dr. M. V. Saradhy, and his Senior Assistant, Mr. R. Narasimhan. There is a lack of precise knowledge of the factors controlling development in the cane plant. Identical response to treatments which are unrelated or even opposite in nature and variance in the effect of the same treatment during different years have been noticed. For instance, the usually non-flowering *S. spontaneum* Burma, recorded favourable response to the 22 hours darkness treatment while later it gave favourable response to 22 hours continuous light treatment. As regards variance in results, early flowering was induced in a previous year, in POJ. 2725 in the 6 hours-day treatment, while in later experiments the same treatment delayed the flowering.

The experiments have now been graded at closer intervals. Photoperiods ranging from ¾ hour to 12 hours both in respect of extra light and darkness and in relation to (1) pre-initiation, (2) initiation, and (3) post-initiation stages of the floral primordia are in progress for the last two years. Sugarcane seems to be very sensitive or exacting in its photoperiod factor. In the pre-initiation stage any deviation of over 1 hour completely inhibits flowering. At the initiation stage the flowering is delayed while in the post-initiation stage there is not much effect. As regards the irrigation and manure factors, profuse irrigation seems to have an accelerating effect on flowering while the nitrogenous and phosphatic manures at the levels (100 lb. Nitrogen, 75 lb. P_2O_5) tried did not have much effect. These experiments are proposed to be continued with graded and increasing manurial doses.

As a pre-requisite to further studies on flowering, observations on the initiation and growth of the sugarcane inflorescence have been made. It has been found that the floral initiation takes place by about the end of August each year and is practically simultaneous in both early and late varieties. Though, as now found, the floral initiation is at about the same time, the factors which make a variety to flower early and another late in the season have yet to be determined. Observations on the growth of the inflorescence indicate that the inflorescence completes its growth in length at the short blade stage and the emergence of the arrow is the function of the elongation of the stalk. The growth of the inflorescence itself appears to be of the auto-catalytic type while the elongation of the stalk is exponential. Negative correlation seems to exist between the elongation of the inflorescence and stalk.

VI. Survey of Manufacturing Qualities

Shri K. V. Gopala Aiyar is in charge of the Chemistry Section and in addition to the rather routine work of analysing the juices of the very large number of sugarcane seedlings under test each year, has recorded valuable observations on the effect of soil conditions on the growth and quality of cane varieties as also the effect of planting in different months of the year on the maturity and growth of sugarcane. He is now conducting for the last four years a survey of the factory areas with a view to gathering information on the manufacturing qualities of the Co. canes in cultivation. This information when completed is proposed to be utilised in selecting suitable parents in the breeding programme so that parents which pass on to their progeny undesirable qualities in the juice may be dropped from the list of parents and only such as possess good qualities retained for crossing work.

There has been considerable improvement in the recovery of sugar per cent. cane obtained by the sugar factories in India in recent years. Side by side with this increase there is also a steady increase in the sucrose per cent. in the cane crushed, while the fibre has remained more or less at 15·50%. It may be said that the improvement in recovery is partly on account of the rise in the technical efficiency of factories and chiefly on account of the improvement in the quality of the Co. canes.

Juices of some varieties have on certain occasions been found to be refractory. In Bombay State difficulty was experienced by some of the factories regarding clarification of juice in POJ. 2878. It was associated with phosphate deficiency in some cases, though not always explained on that score. In Bihar, difficulty was encountered in filtration with the juice of Co. 313. The practice during the recent years has been to study as far as possible the seedlings in the test plots at Coimbatore before they are released to the State Testing Stations for the non-sugars in juice including colloids, ash, phosphoric acid, as also protein and non-protein nitrogen as these are the constituents which will affect the manufacturing qualities.

The non-sugars may well be expected to assume greater importance in the relatively inefficient open pan system of gur manufacture which is a cottage industry consuming more than 60% of the crop. Large varietal differences in gur quality have been noticed and the following juice characteristics would appear to be conducive to good quality gur; (1) a low level of colloidal matter (total colloids and also gums and pectin), (2) a low level of mineral matter, (3) a higher level of phosphates, (4) a smaller proportion of mineral matter present in the colloidal stage, (5) lower values of soluble SiO_2, Fe_2O_3, Al_2O_3 and CaO, and (6) a low level of non-protein nitrogen.

VARIETAL PROBLEM WITH SPECIAL REFERENCE TO ITS PHYSIOLOGICAL AND BIOCHEMICAL ASPECTS

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Although nitrogen-yield index has now been tested over a wide range of soil and climate conditions, its actual use in breeding agrotypes that would make less exacting demand on soil has not been given due attention. Nor has any attempt been made to elucidate the manner in which the low-nitrogen potential operates. Further 'quality' as distinct from 'quantity' has remained obscure and the view is expressed that it is the study of the physiology and biochemistry of leaf tissue that promises fruitful results in respect of capture of solar energy as also its best utilization. Dark green leaf is reckoned to absorb more energy compared to its pale green or green counterpart and stimulus to storage of starch or its simpler derivatives is affected by variety of salts applied as part of nutrient solution. 'Early indicator characters' correlated with different ratings of quality are beginning to be recognised, although quantity still largely eludes reliable forecasting. Recent experimental evidence that quality can be considerably improved by spraying specific fertilizing elements on to the leaf tissue confirm at any rate partially the postulate given above. Data gathered so far are given and need for co-ordinated work on a standardised basis at a number of stations located at representative sites is stressed.

AN AGRO-BOTANICAL PROBLEM AFFECTING THE SUGARCANE INDUSTRY IN INDIA

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For a proper understanding of the suitability of a particular type or variety of sugarcane to its environment and for achieving under a given set of comparatively unalterable conditions the maximum output, it is necessary to take into account certain agro-botanical and physiological characteristics of the type in relation to the environmental factors. Although the basic requirements of the crop are more or less the same wherever it may be grown, and although sugarcane is a fairly adaptable crop, yet, considering that different types of cane are grown in different soil/climatic tracts of the world and the hybrid genetic composition of these is made up of different proportions of different species or ecotypical races, it may well be expected that the reaction of the different varieties to a given environment will be different and not in all cases can we be sure that the type grown is wholly suited to the tract in which it is growing or that it cannot be improved upon.

The subject of the response of the cane crop or plant to various environmental factors has received a great deal of attention in certain countries. Following upon the useful work done in Java and Mauritius on the form, composition and development of the cane plant and crop, the Hawaiian workers have conducted extensive studies on its responses to the environment, even though the differences in the environment obtaining under their insular conditions were perhaps comparatively smaller than in the continental areas. Interesting contributions have also been made to these aspects by other workers. The scope of such studies has been widened in recent years by the work of Brandes and his associates, who have taken more or less a global view of the problem. While the Hawaiian researches were almost entirely directed to increasing the output, the subject is now becoming important in another direction bearing on the adaptation of hybrid-varieties to the environmental conditions of the sub-tropical areas.

An impetus has been given to breeding work for the sub-tropical areas through the discovery of new races and species of plants allied to sugarcane. With the collection of clones of various species of wild and cultivated sugarcane, the prospect of breeding for different soil/climatic

tracts is bound to be greatly enlarged; considering the possibilities of this comparatively little known breeding material against the background of the available knowledge on the responses of cane crop to environmental factors, it is perhaps possible to visualise that the best utilization of these genetic stocks can be made when the differential response of these widely assorted stocks to the diverse environmental factors is studied and understood. It is possible to expect that with such a knowledge, the breeding and selection work can be placed on a more sound footing.

Such a view is perhaps implicit in the recent transplant experiments conducted by Brandes to find out the growth responses of different species and races of plants allied to sugarcane to environmental differences brought about by latitudinal displacements from natural or " normal " latitudinal locations of each one to that of each of the others. In these studies the main factor studied is day-length, a factor which is found to affect not merely the flowering but also the proliferation and growth sequences of the plant. Besides light, however, there are certain other factors such as temperature which affect cane growth not only quantitatively, but also perhaps qualitatively by modifying the proliferation-pattern of the plant and affecting the more intricate processes controlling the activities of the plant.

In a manner of speaking, this problem of the response of different canes to different environments has been ever present in India, where sugarcane has been grown from time immemorial in tracts characterised by marked differences in soil-type, temperature, rainfall, atmospheric humidity and the range of diurnal and seasonal variation which is more in some places than in others. These soil-climatic tracts have also had under cultivation more or less different types and species of sugarcane with marked differences in agro-botanical characters. A considerable amount of information on the agro-botanical differences between these types has been bequeathed to us in the well-known studies carried out by Dr. Barber, but although the differences between the types are interesting and their taxonomic basis has been confirmed to some extent by counts of chromosome numbers, strangely enough their association with the soil-climatic tracts is not very close and neither the association nor its exceptions have been fully understood. Possibly this is due to the effect of human selection and artificial displacements of types through the exchange of clones. Since then these species and types have been replaced by hybrid varieties. The success of these latter has temporarily masked the need for studies on the response of cane to environment and for a knowledge of the agro-botanical characteristics in relation to the climatic tracts. From the varietal aspect, for the immediate needs

of the industry it has been generally sufficient to find a cane and a successively better one which can grow and yield sugar under given combination of favourable and unfavourable environmental factors. But with a basic knowledge of the precise relationship of the characteristics of different types of cane to the various environmental factors, we might not only understand the nature of the genetic stocks better and thus improve our hybrid material, but also make rapid progress in varietal selection and adaptation.

The importance of this problem has been recently 'highlighted' by Leake. To illustrate the diverse response of the cane plant to environmental conditions, he instances the differences in the nature and sequence of growth and proliferation of cane in the tropics and sub-tropics. While much of this difference is known to be accountable to certain specific environmental conditions, it is possible that the various races and species of sugarcane—wild and cultivated—have their own proliferation-patterns as heritable characteristics and in some of the types these characteristics may be more adaptable and plastic while in others they are comparatively rigid. This aspect affects the very basis of crop-production, but fortunately for us adaptability and compensation in cane, where present, help to mitigate the influence of proliferation-patterns. Then again, we have the problem of ripening. It is perhaps possible with our present knowledge to partly understand and explain the differences in the progress of ripening as observed in the different tracts, but the precise relationships between the individual controlling environmental factors, temperature, humidity and soil condition on the one hand and the physiological processes of photosynthesis, translocation, sugar synthesis and growth on the other are not known in a clear and disentangled form from their combined effects and reactions. This is a purely physiological aspect, but since the governing factors are largely beyond human control, the solution of the problem may very largely have to be attempted through a careful choice of the parent material.

Leake conceives of these studies on a world-wide scale, as they have to be if they are to have the advantage of the wide variation in environment under which sugarcane is grown. He stresses the need for the co-operation of research workers of many countries, and the standardisation of methods and measurements. In India the problem is of much importance. We have a sugar industry covering the tropical and the sub-tropical; we have cane cultivation under even more widely varying environmental conditions, in tracts which might well seek the application of useful results, even though they do not have a white sugar industry. While the problem is

thus important, we also have certain advantages. We have a network of research stations where these studies can be carried out. A fairly wide range of genetic stocks of various cultivated and wild canes from within the sub-continent as also from parts of the adjoining countries is now available for study and use in breeding. It should be useful and possible to undertake co-ordinated studies on the responses of various types of cane to the various combinations of environmental factors. While the ultimate results of such studies would lead to well controlled breeding and further refinements in the selection technique, the most immediate results will help the individual tracts themselves to step up their agricultural improvements.

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SOME ASPECTS OF SUGARCANE BREEDING IN RELATION TO ITS CYTO-GENETICAL PECULIARITIES

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The methods followed in sugarcane breeding are necessarily a consequence of its cyto-genetical features. Inbreeding and pure-line selection are almost impossible. The presence of a large accumulation of recessive lethal genes, results in there being practically no survivals in the selfed progeny. Compared to the basic chromosome number 10 for the tribe to which sugarcane belongs, the different species of *Saccharum* are high multiples thereof. *S. officinarum* of which we have a collection of more than 150 varieties are octoploids except Kajla which shows $2n = 68$. In *S. barberi* within which there are four well-defined groups the chromosome numbers vary from 82 to 140. In *S. robustum* the range of chromosome numbers is from 60 to 144. In *S. sinense*, 118 to 122. In *S. spontaneum* it is 54 to 128. Thus the simplest material available shows a high degree of genetical impurity. Orthodox methods of breeding in such highly heterozygous polyploid complexes can be of no avail. While autosyndesis makes segregation along Mendelian expectation almost impossible, it also confers the advantage of wide crossability provided the parental chromosomes are compatible enough to co-exist alongside of one another in the hybrid complement. Such wide crosses with bamboo, *Sorghum*, maize, *Sclerostachya* and *Narenga* are traceable to this phenomenon. The ultimate effect of all this and other features to be mentioned later down, is that there is bound to be a large element of chance in sugarcane breeding which cannot by any means be eliminated altogether.

While these are to be expected in forms of the genetical make-up of sugarcanes, there are certain features which may be regarded as unique some of which are of direct importance in sugarcane breeding. Of these the most recently noticed is the phenomenon of cytoplasmic inheritance (Raghavan, 1951 *a*). Neither a wholly maternal inheritance nor its universality is suggested. There does however, appear to be an interaction between the genes and the plasmon resulting in a configuration leaning more towards the mother than to the father, not easily explicable on the basis of purely genic inheritance. This would appear to explain why in several

hybrids there is very little expression of the male characters irrespective of the number of chromosomes contributed by either parent. For instance recently two seedlings were derived from a cross between sugarcane and bamboo (Raghavan, 1952). This cross was different from those made previously (Venkatraman, 1937), in that for the first time, a pure *officinarum* (var. Vellai) was used as the mother instead of complicated bispecies and trispecies hybrids like Co. or POJ. canes. The gross resemblance of both was to the sugarcane. One was thick and the other thin. The former came into being by the union of a diploid egg with the sperm of bamboo. In the latter it is presumed that an egg with an unexpected number of chromosomes has functioned. Apart from chromosome numbers there are a few characters of the male parent which point to the seedlings being genuine hybrids, like the arched root eyes, the bud piercing through the sheath, underground branching of stem, etc. Even so the hybrids are cane-like. Nor can it be generalised as a rule that the thinness of the second hybrid is due to its having received lesser number of chromosomes from the mother. For cases are known where in the same cross hybrids have come into being having chromosomes $2n$ from the egg and $2n$ from the sperm, and $2n$ from the egg and n from sperm. And yet the general configuration of all the hybrids is more or less similar showing that the dosage effect of certain of the parental chromosomes is very limited. The latest known such case is *S. spontaneum* $\times$ Sweet *Sorghum* ($2n = 74$ and $2n = 84$); in *S. officinarum* var. Chittan $\times$ *Sclerostachya* and *Saccharum robustum* $\times$ *Sclerostachya* both $n + n$ and $n + 2n$ hybrids have been got but with no phenotypic differences (Raghavan, unpublished). On the other hand, there are crosses, where seedlings of a cross, though having the same chromosome number, show different phenotypic expressions, *e.g.*, *S. officinarum* var. Zw. Cheribon $\times$ *S. spontaneum* ($2n = 112$). If, however, a detailed study of the characters and their inheritance is made, it is possible to classify them into three—those which are affected by dosage of genes, those showing complete dominance and those showing no definite pattern of inheritance. Of these the last named is preponderant. These discrepancies can only be explained on the basis of an inheritance which is neither purely gene-controlled nor purely maternal, but a result of an interaction between the genes and the maternal cytoplasm. From this point of view, it is reasonable to bestow more care to the mother than to the father in sugarcane breeding.

Another phenomenon which is likely to play an increasingly important part in sugarcane breeding is parthenogenesis. It is known that when self-sterile or pollen-sterile forms of Co. or POJ canes or selfed are dusted with pollen from distant genera, the latter acts as a stimulus and induces diploid

parthenogenesis. In pure *officinarum* the flowering is so rare and erratic that it has not been studied properly so far. Also the presence of a large number of recessive lethal genes makes survivals few. The exact method of coming into being of an unreduced egg is not known definitely. It is likely to be through endomitosis in the basal spore of the linear tetrad, such as Bremer (1948) has indicated. Narayanaswamy (1940) has said that there takes place a fusion of the two basal spores of the linear tetrad. It has not yet been possible to confirm this finding. In either case, the most important point is that the diploid spore and from it the diploid egg, comes into being not through suppression of meiosis, but is the result of a post-meiotic process, and as such tantamount to fertilisation. So all the vagaries that one can expect in seedlings arising out of fertilisation in a polyploid complex like sugarcane, can be expected in parthenogenetic derivatives as well. And that is what we find. A number of Co. canes having well-known pollen-sterile forms as mother—and generally only such forms are used provided they are otherwise desirable—were examined cytologically and they showed the maternal somatic number indicating their parthenogenetic origin. For example, Co. 462, Co. 463, Co. 656, Co. 779, have all arisen from Co. 421, a pollen sterile form and show only the maternal diploid number 118, even though the staminate parent used in each case has been different. So also Co. 678, Co. 797, Co. 798 and Co. 811 which have presumably arisen parthenogenetically from Co. 603. The very fact of their being different Co. cane numbers is itself proof of the fact that they are widely different from one another. All these variations are a consequence of parthenogenesis being almost a substitute for fertilisation in sugarcane. There are so many chromosomes in the polyploid complex and the pairing autosyndetic, that the resulting phenotypic configurations are many and unpredictable, even though they are all derived parthenogenetically. In addition to diploid parthenogenetic derivatives a few cases are known where daughter seedlings have come into being containing neither the diploid number nor the haploid number. Usually they contain a number less than the diploid number and more than the haploid. The latest known case is that of Co. 602 ($2n = 118$). In selfs of this, we get two types of seedlings, one having the diploid number and the other having $2n = 96$. Obviously 11 bivalents have been eliminated and the remaining 48 bivalents have undergone doubling through endomitosis. The case of *S. officinarum* D. 74 ($2n = 80$) $\times$ *S. spontaneum* ($2n=64$) giving seedlings with 112 and one with $2n = 60$ may also be interpreted on the basis that the former are crosses ($2n + n$), but that the latter are a product of parthenogenesis from an egg with 60 chromosomes. The same is the case with Co. 421 ($2n = 118$) from which in addition to diploid plants, a

few having $2n = 86$ were got (Parthasarathy, 1946; Subramaniam, 1946). It means that not only has chromosome elimination taken place *en bloc* but also side by side, a process of doubling of the functioning group of chromosomes through endomitosis (Parthasarathy, 1951). These two opposing processes of deletion and doubling bring into existence new chromosome races exhibiting a wide range of variability. This is bound to play a very important part in sugarcane breeding. That the sugarcanes are able to withstand such elimination *en bloc* is due to their high polyploidy.

Very recently there was observed a case where two parthenogenetic derivatives from *S. officinarum* var. Striped Vellai, showed a number higher than the diploid number (Raghavan, unpublished). How exactly the egg came to possess this number is not known. But this only makes the problem more interesting and it looks as though that in sugarcane breeding parthenogenesis has played and is playing a more important part than has hitherto been surmised.

There is also evidence of such eggs brought into being through these opposing forces of numerical diminution and multiplication of chromosomes and therefore having neither the haploid nor the diploid number, taking part in fertilisation. Co. 453 a thickish cane, now becoming popular in North India (some parts of Uttar Pradesh and Bihar) is one such (Raghavan, 1951 *b*).

In applying the laws of genetics to sugarcane breeding, one is confronted with the difficulties that one may expect in dealing with a plant of its genetical constitution, *e.g.*, the simplest material available is highly polyploid with an equally high degree of genetical impurity and as such there is generally an absence of Mendelian segregation on expected lines due to autosyndesis. The important genes in sugarcane are those controlling the physiological rather than morphological reaction—sucrose content, disease resistance, etc., and these are characters which cannot be easily identified.

In addition to these inherent difficulties, the special cytogenetical features characteristic of sugarcane of which some idea has been given only tend to make breeding more complicated and unpredictable.

The discovery of an increasingly larger element of chance seems inevitable in sugarcane breeding as more details come to light of its cyto-genetics.

The main problem therefore is to bring sugarcane breeding within the operation of laws of Mendelian Heredity. This can be achieved by reformation of the original but unknown diploid progenitor. With such simplification a high degree of genetical purity may be expected to be restored. One

step towards such a simplification is haploid parthenogenesis of which no sign exists so far. Chromosome elimination *en bloc* such as has been described may perhaps be made use of in some manner. Another way is by continued back-crossing with forms which are likely to have played a part in the origin of sugarcane. This is being done. But the problem is to bring about an elimination of all chromosomes other than those of sugarcane. Studies of the meiotic configuration in such back-crossed progeny have indicated the possibility of *Sclerostachya*, *Narenga*, *Sorghum* and perhaps *Erinathus* having entered into the constitution of the progenitors of *S. officinarum* (Raghavan, 1951 *c*).

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PATHOGENICITY, SPREAD AND REVERSION OF *PHYSALOSPORA TUCUMENENSIS* 'D' RAFAY

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The red rot disease since its first scientific record in Champaran District (Bihar) in 1902 has been recognised as the most severe disease of cane. The control measures recommended by Butler were planting healthy setts and avoiding varieties susceptible to the disease. In 1931 when the disease was found to be very conspicuous on Co. 210 at Pusa the measures advocated by Butler succeeded in the elimination of the disease. Co. 213 replacing Co. 210 in the then limited acreage remained free from the disease for the following eight years. In 1939–40 and the season following Co. 213 also succumbed to the disease. A large number of isolations during the epidemic revealed the occurrence of dark strain 'A' which existed prior to 1939 also, the preponderance of the new light strain 'B' which was responsible for the epidemic and an intermediate form 'C' of rare occurrence which appeared to be more labile than the other two. Susceptibility tests conducted with strain 'B' showed Co. 313 to be less susceptible and Co. 453 resistant. The parasite survived three months in well, river and flood water, seven months in cultures, eleven months in soil and sixteen months in debris. Consequently the control measures advocated were modified to clean setts and clean fields besides the replacement of the susceptible varieties. When Co. 213 was withdrawn the strain continued its existence on Co. 313 leaves and subsequently on Co. 453 leaves without affecting the stalks. The cane-acreage under Co. 453 had reached its peak in 1949 when it assumed a still more virulent form type 'D' and attacked its stalks. The development of a new race of the parasite was sudden and caused concern over the control measures employed. This was neither anticipated nor could it be predetermined. The existence of the disease till 1952 in Motihari, Chakia, Sugauli, Majhaulia, Harinagar, Motipur, Hathua and Sidhwalia reserved areas and Darbhanga and Shahabad Districts demanded further work on the spread of the parasite.

In the current season (1952–53) *Sorghum vulgare* Pers. (Sirsa Var.), *S. halepense*, *Zea Mays*, *Narenga*, *Erianthus munja*, *Saccharum robustum*, *S. fusca*, *S. spontaneum*, *Napier* grass, *E. arundinaceus* and *Arundo donax* were planted or sown between the diseased variety ×1172 affected with strain 'D'. From the diseased setts the disease was transmitted up the

spindle forming lesions on 15th May 1952 and this primary infection alone increased till the end of June 1952. From 1st July 1952 onwards secondary infection on mid-ribs of healthy cane varieties and grasses appeared and progressed. In the post-monsoon period the lesions on cane mid-ribs decreased but increased substantially on grasses, particularly various collections of *S. spontaneum* growing near cane-fields. The rate of spread was thirty yards per week in cane varieties and jowar and only one yard in maize. On clump basis the secondary infection in jowar and *Sorghum halepense* was 100%, *S. robustum* 71%, *Narenga* 66%, *S. spontaneum* 64%, *E. munja* 44%, *S. fusca* 39% and *Napier* grass, *E. arundinaceus* and *Arundo donax* nil. The chances of reinfection of cane are thus immensely increased. Neither seed selection nor seed selection and field selection combined can save the crop from the onset of the parasite harbouring on grasses or susceptible crops. Isolations from the affected mid-ribs yielded strain 'D' except from *S. spontaneum* which yielded the old dark strain 'A' which appears to be due to reversion on congenial host.

The strains are liable to assume aggressive forms on uncongenial and resistant hosts and liable forms may revert to simpler old forms on congenial hosts or chemical media where they have not to strive for existence. The control measures employed should include thorough examination of collateral hosts and their eradication.

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ERRATA

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Page 232, second para, line 7—"one form in Ceylon" *should read* "two forms in Ceylon".

Page 235, line 11 *should read* "Description of the forms".

Vol. XXXVIII, No. 1, July 1953

Article entitled "Thiamine, Its Intermediates, etc.," by K. T. Mathew—*add* under References on pages 8 and 9:

Ramakrishnan, K. and Ramakrishnan, T. S. "Banded leaf-blight of Arrowroot, *Maranta arundi nacea*," *Indian Phytopath.*, 1948, **1**, 129-136.

Rogers, D. P. "The genus *Pellicularia*," *Farlowia*, 1943, 1, 95-118.

Vol. XXXVIII, No. 2, August 1953

Page 100, last para, 4th line from bottom—*for* "liable-' *read* ' labile '.

MICRO-ORGANISMS ASSOCIATED WITH THE "BOMBAY DUCK" OR *BOMBIL* (*HARPODON NEHEREUS* BUCH.)

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Introduction

With incredibly extensive and innumerable shoals, and occurrence practically all the year round, the 'Bombay Duck' or *bombil* (*Harpodon nehereus* Buch.) occupies a conspicuous position among the economically important fishes off the Indian coast. The fish, both fresh and dehydrated, has found an important place in the Indian dietary as such and connoisseurs always appreciate its distinctly fine flavour. Even Europeans had heard about the celebrity of the 'Bombay Duck' through Boswell (1926) the Biographer of Samuel Johnson as far back as the 18th century. Though it is true that the Bombay and Kathiawar shoals are the heaviest—hence the name Bombay Duck—the fish is also plentifully obtained in the estuaries of Bengal and other parts of India. Neither is the distribution of this fish limited to the Indian coast line alone, for it extends from Burma to Zanzibar and even China. However, the mass movements of this fish are fitful, and especially during the monsoons, Bombay is flooded with the heaviest shoals. This brings with it the problem of preservation, and inasmuch as all methods of preservation are primarily aimed at the control of microbial spoilage, a study of the micro-organisms commonly associated with the fish becomes not only apparent but imperative. Added to this, there appears to be no work reported on the microbial flora of this fish, though several workers since Hamilton Buchanan made his first scientific observation to it (1822) have studied its taxonomic, morphological and anatomical details and have even made attempts to study its food and feeding habits as well as the zoological parasites present in the fish (Sethna, 1932; Hora, 1934; Pinto, 1935; Moses, 1946; and Pillay, 1951). The results obtained on studying the micro-flora of this fish are presented in this paper.

Collection and treatment of fish.—The fish utilised for examination was of unquestionable freshness, complying with the tests for freshness recommended by Cox (1946). The samples were collected in a large sterile petri

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dish, which in its turn was placed in another large dish packed with ice, so as to prevent to a considerable extent, any increase in bacterial count during transport. Moreover, the use of a closed sterile petri dish permitted no further contamination by handling, flies, etc. The ice, since it is not allowed to come in contact with the fish, does not further contaminate the same with its own flora. The fresh fish immediately on transportation to the laboratory was thoroughly washed 6 times serially in fresh quantities of sterile water kept in 6 different petri dishes. Other methods employed were (1) the use of 1 : 500 mercuric chloride for 5 minutes as a surface disinfectant, subsequently washing the fish free of the chemical in sterile water, (2) rubbing the exterior of the fish with cotton wool soaked in alcohol and then washing the fish to free it from alcohol prior to dissection and/or analysis. Except for securing the superficial body flora of the fish, where the above treatment was not undertaken, these methods of pretreatment were adopted in all cases and found satisfactory.

Materials for the study of the normal flora.—The methods employed for isolating the flora from various parts of the fish varied according to the nature and location of the part, and the possibilities of its contamination by aerial or other microbes. It must be emphasized here that all dissections undertaken in the process of obtaining the flora from the internal viscera of the fish, were carried out under the strictest aseptic precautions. The contents of the stomach was obtained by directly inserting a blunt probe attached to a sterilized tuberculin syringe, and withdrawing some of the stomach contents, which had been previously flushed with about 0·5 ml. of sterile water. The first drop of the withdrawn suspension was discarded and subsequent drops used for plating procedures on appropriate media. Other methods examined, like suspending incised stomach in sterile water, or boring a hole through the fish with a hot platinum loop to withdraw the stomach contents, were found to be either too laborious or to produce inconsistent results. The flora of the intestine could neither be obtained directly nor as easily as the flora of the stomach because of its position away from the mouth as well as its delicate structure. The walls of the intestine are thin and appear to be turgid and full of fluid when the fish is fresh, whereas in the partially decayed samples this region is usually ruptured and the contents are spilt in the alimentary cavity. Great care is therefore required both in selecting fresh samples as also in the procedure adopted in obtaining the flora from this region. The intestinal region is slightly levered by means of an arrow-head needle and a sterile platinum loop is pierced into the intestinal layer when hot. The heat is sufficient to rupture the wall. The contents are allowed to leak out for some time and then the sterilized platinum wire is inserted a few centimetres

into the intestinal cavity. The contents thus collected, are plated on appropriate media. The flora of the rectum was obtained as in the case of the intestines, and not directly *via* the anus, which present possibilities of contamination. The microflora of the skin and gills were easily examined by preparing suspensions of skin scrapings and incised gills. By adhering to the methods outlined, we have succeeded in getting consistent results and secured a flora which may be said to be normally associated with the fish.

Methods employed for the isolation and identification of micro-organisms.—The selection of various media for the detection of the flora associated with the fish was made very carefully and with due regard to the natural environments of these micro-organisms. At the same time, it was ensured that a nutritionally exacting organism did not escape being isolated. The media usually employed were the following:—

1. Nutrient agar, pH 7 to 7·2 without NaCl.
2. Nutrient agar, pH 7 to 7·2 with 3% NaCl.
3. Fish infusion agar, pH 7 to 7·2 with 3% NaCl.
4. Czapek-Dox-Thom medium of pH 6·6.
5. 5% Blood agar, pH 7·6.
6. MacConkey's agar, pH 7·4.
7. Enrichment media without NaCl.

Inasmuch as some of the media selected are routinely employed in most laboratories, their composition is too well known to require any mention. The composition of other media which are not so well known is given below.

Fish infusion agar

Fish infusion*	1000 ml.
Bacto-peptone	10 g.
Sodium chloride	30 g.
Agar	30 g.
pH	7·0–7·2

Enrichment media.—The basal solution for the various enrichment media was made up as follows:—

Distilled water	1000 ml.
Ammonium sulphate	0·5 g.
Di-potassium hydrogen phosphate	0·5 g.

* For every gram of minced fish, 2 ml. of distilled water were used for extraction in the refrigerator (overnight) and thereafter in the autoclave.

Magnesium sulphate	0·1 g.
Ferrous sulphate	0·02 g.
Calcium sulphate	traces†

The composition of the various enrichment media was as follows:—

Yeast autolysate medium

Basal solution	1000 ml.
Lactose	10 g.
Yeast autolysate	1 g.

Yeast autolysate was prepared as follows and 0·1 g. represents roughly 1 ml. of the liquid preparation. One pound of pressed yeast was suspended in small amounts at a time in 500 ml. of distilled water. The mixture was kept in a flask and incubated at 50° C. for 24 hours, after which it was boiled and alkali added to adjust the pH to 6·4. It was then filtered and sterilized after standardisation.

Bile salt medium

Basal solution	1000 ml.
Lactose	10 g.
Bile salts (Difco)	2 g.
pH	7·0

Sodium desoxycholate medium

Basal solution	1000 ml.
Lactose	10 g.
Sodium desoxycholate	5 g.
pH	7·0

The principal methods employed for estimating the abundance of micro-organisms in the fish, both fresh and dehydrated, were direct microscopic counts and plating procedures. Each of the various media enumerated succeeded in eliciting only a part of the microflora of the fish, though collectively they appear to satisfy the growth requirements of a wide range of micro-organisms. The incorporation of 3% salt to various media produced a noticeable increase in count. As an alternative to the addition of salt, it has been suggested that the particular medium be prepared in sea water. Though such a medium is recommended (Berkeley, 1919; Lipman, 1926), in our experience the incorporation of 3% NaCl serves equally well the purpose. A 3% salt agar was selected to represent the marine environment and the

† 1 ml. of a saturated solution of $CaSO_4$ was the amount used.

yeast autolysate enrichment medium was primarily chosen to detect the presence of moulds and yeasts. Fish infusion agar and 5% blood agar were chosen to correspond more closely to the nature of fish and animal tissue. MacConkey's agar and the other enrichment media facilitated the growth of intestinal organisms, and as will be noted later was to a great extent beneficial in the detection and study of the colony characters of micro-organisms found in the ventriculus, intestines and rectum of the fish, as well as in certain epidemiological studies on the fish conducted in this laboratory.

Results

A microbiological examination of 42 *bombils*, both fresh and dehydrated, according to the methods already described, revealed a flora which was consistent enough to be regarded as normal. These bacteria, being marine in origin, differed in certain respects from their terrestrial counterparts, notably in cell morphology, certain cultural and physiological characteristics, and salinity requirements.

Cell morphology.—A large percentage of the bacteria found on the fresh *bombil* were gram negative rods, capsulated in the animal body, but losing this property on artificial cultivation. Collectively, this group is not morphologically differentiable, and pleomorphism is quite pronounced in most of them. A comparatively smaller proportion of the bacterial flora was made up of cocci, gram positive rods, and sporeforming bacilli. From the ventriculus of the commercially dehydrated fish, the flora appeared to be more characteristic.

Cultural and physiological characteristics.—In general, bacteria from the *bombil* grow more slowly, producing smaller colonies than their terrestrial counterparts from soil, sewage, milk, etc. The colonies on agar are often depressed and those from the alimentary canal are mostly non-pigmented and translucent while surface organisms appear both pigmented as well as non-pigmented. Opacity of the colonies was a variable character. Inasmuch as the habitat of *Harpodon nehereus* is tropical waters, which according to ZoBell (1946) have surface temperatures as high as 28% C. to 30° C., or 38 to 40° C. in localized regions near shore, it is not surprising that with regard to the microflora of the *bombil*, moderately good growth was observed at room temperature (28 to 30° C.). Although ZoBell and Grant (1943) present evidence which suggests that all heterotrophic marine bacteria are able to assimilate glucose, only 46 of the 60 cultures studied by ZoBell and Upham (1944) fermented glucose with the formation of acid and none of them produced gas from glucose. The normal micro-flora of the intestine, ventriculus and rectum of fresh *bombil* present evidence to the contrary. These

micro-organisms isolated are as strongly saccharolytic as they are proteolytic. Practically all of them fermented with 'acid and gas', besides fermenting in a like manner, maltose, saccharose, starch and dextrin. Formation of hydrogen sulphide from 2% peptone salt water in 24 hours was a property common to nearly all these cultures. Likewise almost all isolates reduced nitrates to nitrites. However, these bacteria were conspicuous by their inability to produce indol from tryptone salt water and to ferment lactose. A frequently occurring gram negative coccobacterium which is remarkably heat resistant and persists even in the dehydrated fish, was observed to be physiologically inactive. Besides those mentioned, there occur other bacteria whose occurrence appear fitful and hence may be classified as a group of adventitious flora of the alimentary canal. Briefly speaking, the following genera have been encountered in the various regions.

Fresh fish

Alimentary canal.—Bacterium, Escherichia, Achromobacter, Pseudomonas and *Flavobacterium.*

Skin, Gills and Flesh.—Micrococcus (also *Gaffkya* and *Sarcina* species), *Bacillus, Escherichia, Aerobacter, Bacterium, Achromobacter.*

Dehydrated fish

Ventriculus or Stomach.—Micrococcus, Sarcina, Bacillus.

We have encountered considerable difficulties in placing these organisms isolated from the *bombil*, in Bergey's (1948) classification. Similar difficulties have been met with by Thjotta and Somme (1943), ZoBell and Upham (1944) and other workers who have been familiar with marine bacteria. We have accordingly recognized slight differences in character of the bacteria isolated only as strain differences and have preferred to refer them to the nearest species in Bergey's (1948) Manual.

Bacterial flora of the fresh bombil.—Though the bacterial flora of the *bombil* varies with the region of the fish examined, certain types of organisms are common to most, if not all, of the regions examined, and this more than justify their claims to be regarded as the normal flora of the *bombil.* Among these, a species of *Sarcina, viz., Sarcina littoralis* and a near-luminiscent non-spore-forming organism which closely answers to the description of *Bacterium phosphoreum* deserve special mention, as they or their variants have been isolated in large numbers in all regions of the alimentary tract of the fish. In addition to these normally occurring species, an adventitious flora made up mostly of *Achromobacter* and *Bacterium* species also occur in the ventriculus and the rectum, but not in the intestines which appear to be free from an

adventitious flora. *Escherichia* (*E. freundi*) and *Aerobacter* species have also been encountered, but only in fish taken near shore. Other isolates obtained from the fresh fish include those of *Micrococcus* species, *Bacillus* species (*B. cereus* and others), *Pseudomonas ovalis* and *Vibrio percolans*.

Though there has been seldom any consistency about the microflora of the surface of the fish, species of *Aerobacter*, *Escherichia* (*E. intermedium*), *Achromobacter* (*A. delicatulum*, *A. superficiale*), *Flavobacterium* (*F. lutescens*), *Bacterium* (*B. mutabile* and others), *Micrococcus* (*M. caseolyticus*, *M. epidermidis*), *Sarcina*, *Gaffkya*, and *Bacillus* have been isolated and identified from the gills, skin and flesh of fresh samples of fish.

Bacterial flora of the dehydrated bombil.—Unlike the micro-organisms present in the ventriculus of the fresh fish, those present in the same region in the dehydrated form have a characteristic flora of their own. Species of *Micrococcus* seem to be very well represented and among the more common micrococci isolated and identified were *M. candidus*, *M. freudenreichii*, *M. epidermidis*, *M. aurantiacus*, *M. flavus*, *M. ureæ*, and *M. albus*. *Sarcina littoralis*, *Achromobacter delicatulum* and two species of unidentified bacilli were also isolated from the dehydrated *bombil*.

Discussion

On the basis of morphological and anatomical details, the *bombil* (*Harpodon nehereus*) is classified among the deep sea fishes, though its shoals are found in abundance along the coast. It has been shown that the skin and alimentary canal of the *bombil* have a characteristic flora of their own and that this flora is not only stenohaline in nature but possess powerful enzymes which apparently are responsible for the spoilage of the fish, the moment it dies. The micro-flora of the live fish and the dead one of course, vary to an extent depending upon the environments, conditions under which they are transported and handled, as also upon the death-examination interval. The occurrence of the colon group of organisms in the ventriculus of fresh fish collected from harbour waters, is significant from an epidemiological standpoint. But for the organism resembling *Sarcina litto alis*, the flora of the dehydrated fish is characterised by the complete absence of bacteria associated with the fresh *bombil*. The presence of *Sarcina littoralis*, even in dehydrated fish, suggests the resistant nature of this organism to adverse conditions like heat treatment and ultra-violet radiation. The frequent occurrence of species of *Micrococcus* (many of which secrete enterotoxins) in the dehydrated form of the fish, stresses the possibilities of this fish being a potential source of food poisoning.

SUMMARY

The fresh ' Bombay Duck ' (*Harpodon nehereus*) has a normal bacterial flora of its own, among which *Bacterium phosphoreum* and *Sarcina littoralis* or their variants predominate. The latter species also persists in the dehydrated variety of the fish, which otherwise presents a microbial picture, totally different from that of fresh fish. Besides the species mentioned, the fresh fish is also associated with an adventitious flora, in which the following genera are represented: *Escherichia*, *Aerobacter*, *Micrococcus*, *Gaffkya*, *Achromobacter*, *Flavobacterium*, *Bacillus* and *Pseudomonas*. The occurrence of a large number and variety of micrococci in the dehydrated fish has a probable epidemiological significance.

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FACTORS OTHER THAN ACTIVE RELAXATION RESPONSIBLE FOR THE LENGTHENING OF UNLOADED UNSTRIATED MUSCLE

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Unloaded unstriated muscle relaxes under certain conditions (Singh and Singh, 1948 *a*, 1949 *a*, 1950 *a*, *b*, 1951 *a*, *b*, *c*, 1952 *a*; Singh, 1951). If adrenaline is added to saline in which are immersed unloaded transverse pieces of the stomach muscle of the frog *Rana tigrina*, or virgin guinea pig's uterus, they lengthen; unloaded dog's stomach muscle relaxes after stimulation with electric current and chemicals. This might be due to two causes: (*a*) active relaxation, (*b*) something pulling it out, such as elasticity of connective tissues or membranes, etc. If the latter explanation is correct, it means the muscle is loaded and relaxation really passive.

There are certain observations which show that relaxation in the above instance is active (Singh and Singh, 1952 *a*):

(1) Unstriated muscle, if loaded, and the load exceeds a certain minimum, lengthens (Winton, 1930). If a force was always tending to pull it out, then the unloaded muscle should gradually lengthen when immersed in saline; but what happens is the opposite, so that any such force does not appear to be operative.

(2) Cyanide abolishes the relaxation, which is again partially restored with glucose (Singh and Singh, 1951 *b*). This shows that during relaxation metabolic processes are involved.

(3) An interesting property of unstriated muscle is that under certain conditions, loaded muscle lengthens, but not the unloaded one (Singh and Singh, 1952 *a*).

In the present research, this last property has been investigated.

Experimental

Two sets of experiments were performed. In the first set, the effect of asphyxia (by hydrogen) or sodium cyanide (1 in 10,000) on the normal tone of loaded and unloaded muscles was studied. In the second set, potassium contraction was first induced, and then the muscle asphyxiated or treated with

cyanide. The length of the unloaded muscle was measured before and during asphyxiation with hydrogen or after treatment with cyanide.

RESULTS

Effect of asphyxia or cyanide on normal tone.—These experiments were performed on dog's stomach muscle at various temperatures from 20 to 37° C., on frog's stomach muscle from 20 to 30° C., on fowl's gut from 20 to 37° C. and on guinea pig's uterus at the same temperatures. The length of the unloaded muscle was measured before asphyxiation by hydrogen or treatment with sodium cyanide (1 in 10,000), and at 5 minutes' intervals after beginning of asphyxiation or treatment with cyanide. This is important because loaded muscle may show contraction after preliminary relaxation on asphyxiation or treatment with cyanide (Singh, 1949), and if the observations are made at long intervals, then this preliminary relaxation might be missed.

The loaded and unloaded muscles differ markedly in their behaviour on asphyxiation or treatment with cyanide at different temperatures; the former relaxes readily at high temperatures, and the latter may do so only at low temperatures (20° C.). At temperature of 25° C. (10 experiments), 30° C. (10 experiments), 37° C. (10 experiments), unloaded dog's stomach muscles did not relax at all; they rather contracted slightly. Loaded muscles relaxed readily and then tended to contract. At 20° C., in 14 out of 34 experiments, unloaded muscles relaxed by about 2 to 20 p.c.; loaded muscles also relaxed at this temperature, but less readily than at higher temperatures. Unloaded frog's stomach muscle did not show any significant relaxation at 20° C. (12 experiments), 25° C. (12 experiments) and at 30° C. (12 experiments); loaded muscle relaxed readily. Unloaded fowl's gut relaxed by 20 p.c. in one out of 12 experiments at 37° C., and did not show any significant relaxation at 30, 25, and 20° C.; loaded muscle relaxed profoundly, especially at 37° C. Unloaded guinea pig's uterus did not show any relaxation at 20, 25, 30 and 37° C. (2 experiments each); loaded muscle relaxed readily.

Effect of iodoacetic acid on normal tone.—Sodium iodoacetate (1 in 10,000), causes relaxation of fowl's gut, frog's stomach, guinea pig's uterus at 37° C. if loaded, but not if unloaded (6 experiments each). Dog's stomach muscle also relaxes if it shows lactic tone.

Effect of temperature.—Gradual lowering of temperature from 37° C. to 25° C. or lower, causes relaxation of dog's stomach muscle, guinea pig's uterus, fowl's gut and human appendix if loaded, but not if unloaded. Frog's stomach muscle behaves similarly if the temperature is lowered from 30° C. to about 10° C.

Sodium azide also caused relaxation of loaded frog's stomach muscle, but not that of unloaded muscle at 25° C. (6 experiments).

Effect of asphyxia on the potassium contraction.—In dog's stomach muscle, tone was increased by replacing 40 p.c. of the sodium of the saline by potassium, and the above experiments were repeated. On asphyxiation or treatment with cyanide, loaded muscles relaxed but the unloaded ones did not do so at 37, 30 and 25° C. (12 experiments each). At 20 to 22° C. a few unloaded muscles also relaxed (Table I); loaded muscles also relaxed, but less readily than at higher temperatures.

TABLE I

Dog's stomach muscle. Effect of asphyxia (by hydrogen) and sodium cyanide (1 in 10,000) on the contraction produced by replacing 40 p.c. of the sodium of the saline with potassium

No. of experiments	Temperature ° C.	Length in saline mm.	Length in potassium mm.	Length during asphyxia of duration			Length after aeration	Length in cyanide		
				5 min.	10 min.	15 min.		5 min.	10 min.	15 min.
1	20	59	55	55	55	56	56	55	55	54
2	20	44	40	39	39	39	39	39	39	39
3	20	41	39	39	39	39	39	39	39	39
4	21	56	49	50	48	45	46	46	46	46
5	21	53	50	52	53	53	54	53	53	53
6	21	55	52	..	..	..	..	55	55	..
7	22	49	44	44	45	45	45	45	45	43
8	22	48	42	42	42	42	42	42	42	41
9	22	43	41	..	..	..	..	42	42	42
10	22	56	53	54	54	54	54	53	53	52
11	22	35	32	32	32	32	32	32	32	32
12	22	45	42	..	..	..	..	42	42	42
13	22	40	38	..	..	..	..	39	39	39
14	22	43	40	..	..	..	..	42	42	42
15	22	55	49	49	49	49	50	50	46	47
16	22	33	30	..	..	..	..	30	30	30
17	22	41	39	..	..	..	..	39	39	39
18	22	51	42	..	..	..	..	43	44	44
19	22	41	39	..	..	..	..	39	39	39
20	22	42	39	..	..	..	..	38	38	38

DISCUSSION

These experiments clearly reveal that relaxation in unstriated muscle is of two kinds, one active and the other passive. Active relaxation is

decreased by all agencies that diminish metabolism; passive relaxation is affected oppositely. There must be, therefore, two systems in unstriated muscle, in one the relaxation being active and in the other passive (Singh and Singh, 1950 *b*, 1951 *d*, 1952 *b*; Singh, 1952, 1953). Relaxation of unloaded unstriated muscle at 20° C., must therefore be due to cessation of stimulation (Singh and Singh, 1952 *a*). These experiments therefore show that in unstriated muscle, both contraction as well as relaxation are energised. The metabolic mechanisms for contraction and relaxation are different, and they are differentially suppressed by cyanide. If the metabolic mechanism for contraction is suppressed, then the loaded muscle relaxes actively as well as passively, and the unloaded one only actively. If the metabolic mechanism for relaxation is suppressed also, then only the loaded muscle relaxes passively; active relaxation is absent. It is not possible to say whether cyanide can suppress only the mechanism for active relaxation; under such conditions, the muscle will relax neither actively nor passively on the addition of cyanide.

If both the systems are active at the same time, then the muscle would be contracting and relaxing at the same time. This should be distinguished from accommodation to the stimulus. Previously, we have described the inhibitory action of glucose on the mechanical response of unstriated muscle (Singh and Singh, 1948 *b*, 1949 *b*, 1950 *c*); this has been ascribed to the antagonism of glycolytic and non-glycolytic metabolic mechanisms. It is more likely due to increase of active relaxation during contraction. If this explanation is correct, then the inhibitory action of glucose should be present only in those muscles, which show active relaxation. Agencies that depress active relaxation would therefore enhance the mechanical response; this they may do so, also by diminishing accommodation (Singh and Singh, 1952 *c*).

Substances that diminish accommodation, also decrease the inhibitory action of adrenaline. These substances also antagonise active relaxation (Singh and Singh, 1949 *a*). It is therefore probable, that the inhibitory action of adrenaline in some muscles is due to active relaxation during contraction; this inhibitory action of adrenaline is therefore due to the contractile mechanism. Adrenaline may also inhibit through the excitatory mechanism only, as rabbit's gut, though very sensitive to adrenaline, does not relax actively.

When a muscle is contracting, its mechanical response is affected by three agencies: (1) the excitatory and the contractile processes (which cannot be easily dissociated); (2) active relaxation; (3) accommodation. Substances that stimulate metabolism will increase the mechanical response by increasing

the first process; substances that depress metabolism will do so by decreasing the second and third processes. Similarly, the metabolic stimulants will decrease the response by stimulating the second and third processes.

The two systems in unstriated muscle are probably represented by two different bonds in actomyosin. Bonds responsible for passive relaxation are dissolved by salts, nad those for active relaxation, are dissolved by urea. The former bonds are modified to produce twitch and lactic tone respectively. The latter bonds are modified to produce alactic tone and tone which is decreased by glucose or oxygen. There may still be further subdivisions to account for the various contractions of unstriated muscle.

Summary and Conclusions

(1) Substances that depress metabolism suppress relaxation of unloaded muscle and increase that of loaded muscle.

(2) It is therefore concluded that relaxation in unstriated muscle is of two kinds, one active and the other passive.

(3) Substances that increase metabolism may diminish the mechanical response, by simultaneously increasing active relaxation, while the muscle is contracting.

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A COMPARISON OF THE CONTRACTILE SYSTEMS OF STRIATED AND UNSTRIATED MUSCLES

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The contractile systems of striated and unstriated muscles are undoubtedly different. The former contains more myosin and actomyosin than the latter (Csapo, 1948, 1950). The high energy phosphate content of unstriated muscle is also much less than that of striated muscle (Csapo and Gergely, 1950); the sustained tension of unstriated muscle must have, therefore, different energetic basis. Striated muscle shows *rigor mortis* ; unstriated muscle does not show this as a terminal event, but one of its normal physiological contractions is akin to it (Singh, 1952; Singh and Singh, 1952). There are well-known histological and physiological differences between the two muscles.

Previously, the effect of substances on the contractile mechanism of unstriated muscle has been described (Singh and Singh, 1949 *a*, 1950 *a*, 1951). In this method the muscle is allowed to die, while immersed in various unoxygenated solutions. It is well known that the permeability of the muscle is maintained by metabolism, so that as the muscle dies, it becomes inexcitable and permeable; the substances then penetrate the muscle and act on the contractile mechanism without interference from the excitatory mechanism. In previous experiments, the excitatory system was also rendered inoperative by high voltages of alternating current or chemicals such as methyl alcohol and chloroform (Singh and Singh, 1951; Singh, 1953).

In the present research, these experiments have been extended to frog's striated muscle, and the differences between the behaviour of the two kinds of muscles, noted.

Experimental

Pairs of sartorii and recti were removed from the frog, *Rana tigrina* ; one of each pair was immersed unloaded in unoxygenated Ringer's solution and the other in the experimental solution for 24 or 48 hours. The sartorii are more suitable than the recti. The length and weight were recorded before and after immersion. Though the effect of various substances on the contractile mechanism of unstriated muscle have already been determined, in

the present experiments, it was redetermined to serve as a control for striated muscle.

Results

The effect of following substances was tried: 0·112 *M* sodium chloride, sodium bromide, sodium iodide, sodium thiocyanate, sodium cyanide, potassium chloride, ammonium chloride, lithium chloride, hydrochloric acid, sodium hydroxide, and isotonic solutions of calcium chloride, strontium chloride, magnesium chloride, barium chloride and urea. The results are shown in Table I. The chief difference between the frog's stomach muscle and striated muscle is that in the former, sodium cyanide and barium chloride cause active relaxation. But the effect of ions on striated muscle resemble those on *Mytilus* unstriated muscle, in which sodium cyanide and barium chloride cause contraction (Singh, 1952). The contractile mechanism of striated muscle of frong, therefore, does not resemble that of unstriated muscle from the same animal but that of *Mytilus* muscle.

Table I

The effect of various substances, isotonic with 0·112 *M NaCl on dying frog's striated muscle (sartorius and rectus) and frog's stomach muscle Average of* 6 *observations* 24 *hours' immersion*

Substance	Striated muscle		Unstriated muscle	
	Percentage increase in length	Percentage increase in weight	Percentage increase in length	Percentage increase in weight
NaCl	−17	20	−11	20
NaBr	−14	23	0	7
$NaNO_2$	−24	17	−68	30
NaI	−19	34	− 5	19
NaSCN	−26	27	−12	28
NaCN	−30	27	38	86
LiCL	−12	25	18	9
NH_4Cl	3	41	5	44
KCl	1	40	14	58
$CaCl_2$	−36	− 8	−12	− 9
$SrCl_2$	−46	−13	6	−10
$BaCl_2$	−48	−21	16	5
$MgCl_2$	1	15	38	39
HCl	3	83	57	56
NaOH	25	7	117	49
H_2O	1	43	114	113
Urea	− 7	42	137	80

Relaxation in striated muscle.—Ramsey and Street (1940, 1941) working on isolated single muscle fibres reported that relaxation was active. Hill (1949) found by a very sensitive method, that relaxation is striated muscle was passive. Relaxation of frog's striated muscles has been studied after they were made to contract with 0·1 *M* potassium chloride or 1 in 100,000 acetylcholine; a preliminary report of these experiments have been published previously (Singh and Singh, 1950 *b*). In 12 experiments each, it was found that the frog's rectus did not relax after it was made to contract with potassium chloride or acetylcholine. This is in marked contrast to dog's stomach muscle, which relaxes actively after such treatment (Singh and Singh, 1949 *b*).

DISCUSSION

The contractile mechanism of frog's striated muscle does not resemble the unstriated muscle in the same animal but surprisingly, it resembles that of *Mytilus* muscle.

Active relaxation has been found in *Mytilus* muscle (Wiersma, 1952). We were not able to find active relaxation in this muscle, so that it apparently formed an exception to other unstriated muscles; we therefore concluded, that its tonic mechanism had a different basis. Our failure to dissociate the tonic and phasic systems in *Mytilus* muscle by mechanical disruption (Singh, 1952), must have been due to damage to both the systems, owing to the comparatively delicate nature of the muscle. The tonic mechanism of *Mytilus* muscle is probably due to actomyosin bonds, the dissociation of which produces active relaxation. These bonds therefore, are different from those of other unstriated muscles, as barium chloride and sodium cyanide do not produce active relaxation of the contractile mechanism of *Mytilus* muscle. The actomyosin bonds which dissociate to produce active relaxation of unstriated muscles, are therefore, of three kinds: (1) Those which can be dissociated by glucose or oxygen. (2) Those which cannot be so dissociated. (3) Those which cannot be dissociated by barium chloride or sodium cyanide.

SUMMARY AND CONCLUSIONS

1. The effect of various substances on the contractile mechanism of frog's sartorius and rectus has been studied by using the dying muscle.

2. It has been found that the effect of various substances on the frog's striated muscle resembles that on *Mytilus* unstriated muscle and not that on frog's unstriated muscle.

3. The common feature of frog's striated muscle and *Mytilus* muscle is, that active relaxation is absent in these muscles.

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ASCOMYCETES FROM SOUTH INDIA—I

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In this series of papers it is proposed to describe new species of Ascomycetes as well as to record old species which are collected for the first time from South India (Madras, Mysore, Travancore-Cochin and Coorg). In the present paper four new species of Ascomycetes are described.

1. *Chætomium rufum* sp. nov. (Fig. 1, A–E).

Perithecia bright purplish red, minute, without scirrih or rhizoids, $110\times92\,\mu$; lateral hairs sparse, septate, slightly curved or once coiled at the apex; terminal hairs numerous, gracefully arched from base to apex, coiled at the apex in one to three spirals, plainly but distantly septate, slightly broadened at base, about $6\cdot5\,\mu$ in diameter in the middle, incrusted throughout; asci clavate, hyaline, $32–45\times8–13\,\mu$; ascospores irregularly biseriate in the ascus, lozenge-shaped, but oval in face view, pale olivaceous brown in colour, 9×6 ($8–10\times5–8\,\mu$), released into the perithecium by the deliquescence of the ascus wall.

Growing on wet straw, University Botany Laboratory Campus, Madras, 28–2–1953, coll. K. Ramakrishnan [Herb. M.U.B.L. No. 866 (Type)].

Perithecia micanter purpureo-rubra, minuta, absque cirrhis vel rhizoideis, $110\times92\,\mu$; pili laterales sparsi, septati, tenuiter curvati vel semel spiraliter curvati ad apicem; pili terminales plurimi, perpulchre arcuati ex basi ad apicem, spiraliter curvati ad apicem in unam vel tres spiras, simpliciter sed distanter septati, tenuiter dilatanti ad basim, ca. $6\cdot5\,\mu$ diam. ad medium; per totam longitudinem incrustati; asci clavati, hyalini, $32–45\times8–13\,\mu$; ascospores irregulariter biseriatae in asco, rhomboideæ, sed ovatæ in aspectu faciali, pallide olivaceo brunneæ, 9×6 ($8–10\times5–8\,\mu$); cito solutae in perithecium post deliquescentium parietalis ascalis.

Typus lectus in stramento madido, in Campo University Botany Laboratory, Madras, a K. Ramakrishnan, die 28 februarii 1953, et positus in herbario M.U.B.L. sub numero 866.

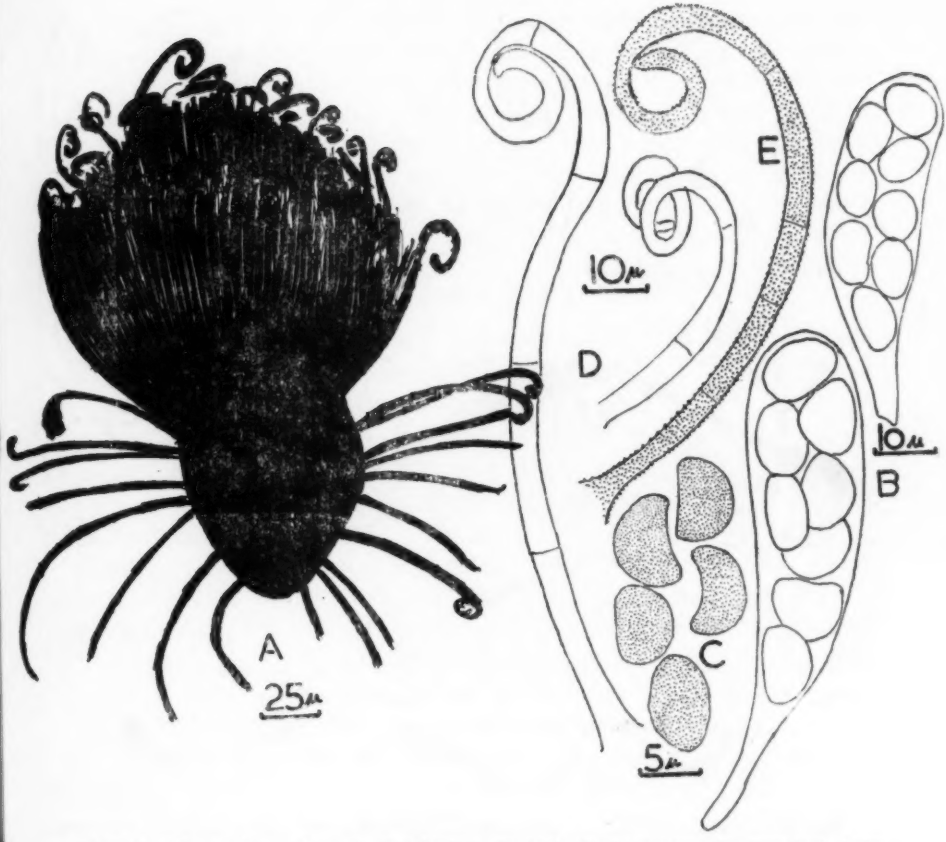

FIG. 1. *Chaetomium rufum*, A. Perithecium, B. Asci, C. Ascospores, D. Terminal hairs, E. One terminal hair showing the incrustation.

As far as the author is aware the only species of *Chætomium* described so far with lozenge-shaped ascospores is *C. trilaterale* Chivers (Chivers, 1915, p. 175). This species, however, has lemon yellow perithecia which are slightly smaller than those of the present fungus. The terminal hairs are minutely roughened unlike those of the present fungus which are conspicuously incrusted.

2. *Polyclypeolum salvadoræ* sp. nov. (Fig. 2, A–F)

Foliicolous; spots none, scutella amphigenous, superficial, black, minute, isolated or confluent with one another in lines; lower wall of scutella indistinct; upper wall black, made up of dark brown cells which appear

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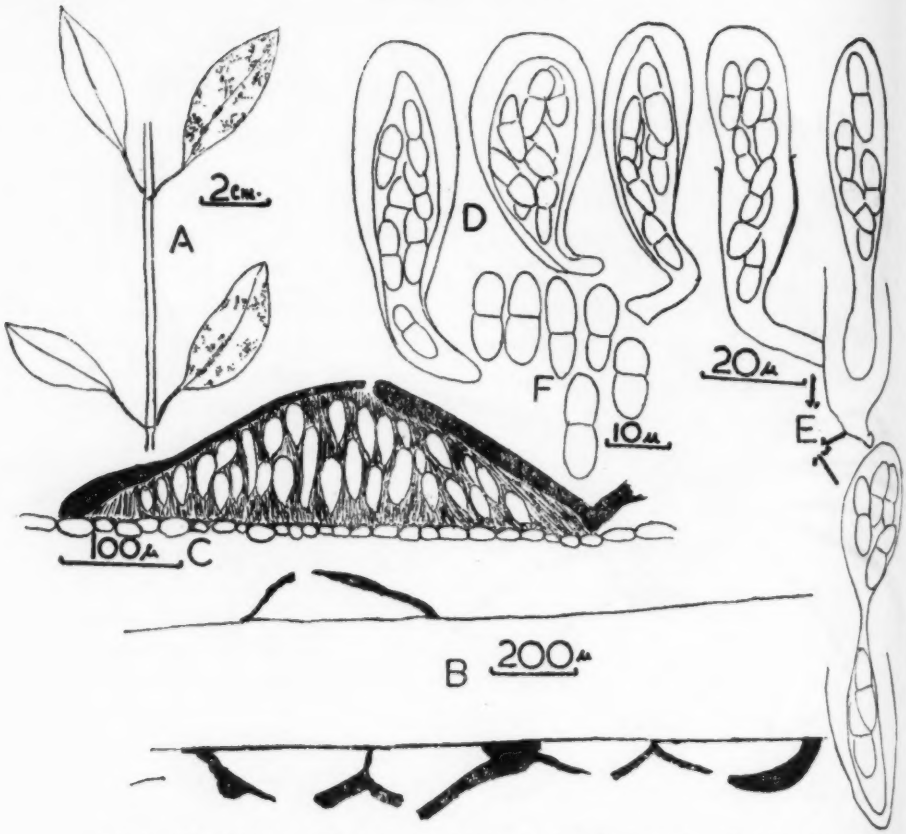

FIG. 2. *Polyclypeolum salvadoræ*, A. A twig of *Salvadora persica* showing two of the infected leaves, B. Cross section of leaf showing the amphigenous scutella (diagrammatic), C. Section through one scutellum (diagrammatic), D. Asci, E. Asci, showing the process of dehiscence, F. Ascospores.

radial at the margins, elevated hemispherically over the locule, without an ostiole; loculi hemispherical; hymenium of two to three irregular layers, with asci separated by areas of sterile stroma; asci hyaline, stout, broadly club-shaped with an attenuate base and rounded apex, bitunicate, inner wall very thick especially at the apex, outer wall very thin and membranous; asci 48–67 × 19–26 μ; ascospores hyaline, two-celled, constricted at the septum, elliptical, 14·4–20·8 × 4·8–8·6 μ.

On living leaves of *Salvadora persica* Linn., Ennore (Chinglepet District, Madras State), 29–3–1953, coll. T. S. Sadasivan [Herb. M.U.B.L. No. 885 (Type)].

Foliicola; maculæ nullæ; scutella amphigena, superficialis nigra, minute, solitaria vel confluens cum aliis in lineas; inferior paries scutellæ indistinctus, superior vero niger, compositus cellulis fusce brunneis, quæ ad margines videntur radiales, hemispherice elevatæ supra loculam, absque ostiolo; loculi hemispherici; hymenium constans duplici vel triplici serie irregulari, ascis separatis inter se per areas stromatis sterilis; asci hyalini, robusti, late clavati, basi attenuata, atqu apice rotundato, bitunicati; inferior paries crassissimus prasertim ad apicem; exterior vero paries tenuissimus atque membranaceus; asci 48–67×19–26 μ; ascosporæ hyalinæ, bicellulatæ, constrictæ ad septum, ellipticæ, 14·4–20·8×4·8–8·6 μ.

Typus lectus in foliis viventibus *Salvadoræ persicæ* Linn. in loco Ennore (Chinglepet District, Madras State) die 29 March 1953 a T. S. Sadasivan et positus in Herb. M.U.B.L. sub numero 885.

It has not been possible to observe the actual dehiscence of an ascus. However, observations made on several dehisced asci suggest that the dehiscence of the asci is as in *Myiocopron smilacis* (De Not.) Sacc. and *Ellisiodothis inquinans* (E. and E.) Theiss as described by Luttrell (1944 and 1948 respectively). The outer wall of the ascus which is very thin bursts and then the inner wall elongates using up the very thick wall material at the apex and the sides (Fig. 2 E). The release of the ascospores was not observed.

There seems to be some doubt about the definition of the genus *Polyclypeolum*. This genus was described by Theissen (1914, and Saccardo, 1926, p. 527) for a fungus with many-layered hymenia of asci. The ascomata were described as aparaphysate and astomous. Hansford (1945), however, described a species of the fungus-*P. brideliæ* Hansford from Ugunda in which he mentioned numerous paraphyses. I have followed the diagnosis of Theissen in assigning my fungus to the genus *Polyclypeolum*.

3. *Phyllachora kambakkamensis* sp. nov. (Fig. 3, A–C)

Foliicolous; spots amphigenous, irregular in outline, yellowish with a tinge of red; stromata visible on both sides of the leaf, scattered or in irregular groups, raised slightly over the surface of the leaf, black, shining, minute, 0·25–0·5 mm. in diam., roughly circular, occupying the entire mesophyll of the leaf; loculi one in each stroma, 140–160 μ in diam.; asci hyaline, 8-spored, clavate with a slight foot at base, 55–67×9·6–12·8 μ; ascospores hyaline, oval, uniseriate in the ascus, 12×5·6 μ (9·6–12·8×4·8–6·4 μ); paraphyses numerous, hyaline, simple.

On living leaves of *Ochna* sp., Thanthipandal (Kambakkam, Chinglepet District, Madras State), 3–2–1952, Coll. C. V. Subramanian and K. Ramakrishnan [Herb. M.U.B.L. No. 758 (Type)].

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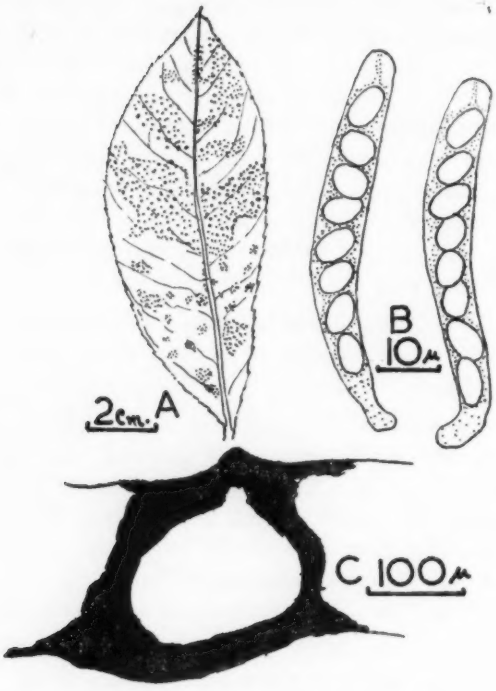

FIG. 3. *Phyllachora kambakkamensis*, A. A leaf of *Ochna* sp. showing the stromata B. Asci, C. Section through one stroma showing one loculus (diagrammatic).

Foliicola; maculæ amphigenæ, irregulares, luteolæ sed rubriuscule tinctæ; stromata visibilia in utroque lateræ foliorum, dispersa vel irregulariter aggregata, tenuiter elevata super foliorum superficiem, nigra nitenttia, minuta, 0·25–0·5 mm. diam.; plusminusve circularia, totum mesophyllum foliorum occupantia; loculi singuli in singulis stromatibus, 140–160 μ diam.; asci hyalini octospori, clavati, ornati tenui pediculo ad basim, 56–67× 9·6–12·8 μ; ascosporæ hyalinæ, ovatæ, uniseriatæ in asco, 12×5·6 μ (9·6–12·8×4·8–6·4 μ); paraphyses plurimæ, hyalinæ, simplices.

Typus lectus in foliis viventibus *Ochnæ* sp. in loco Thanthipandal (Kambakkam, Chinglepet District, Madras State) a C. V. Subramanian et K. Ramakrishnan, die 3 februarii 1952, et positus in Herb. M.U.B.L. sub numero 758.

Phyllachora ochnæ Pat. and Har. has been described from the Philippines (Saccardo, 1926, p. 606). This fungus produces amphigenous stromata which

are circularly arranged. The asci are $60 \times 12\,\mu$ and the ascospores $24 \times 5\,\mu$. It would be seen that the present fungus differs from *P. ochnæ* in the very much smaller size of the ascospores and the slightly larger asci.

4. *Phyllachora tetraspora* sp. nov. (Fig. 4, A–D)

Foliicolous; spots none, stromata visible on both sides of the leaf, either scattered, in groups of two or three or arranged in circles around a

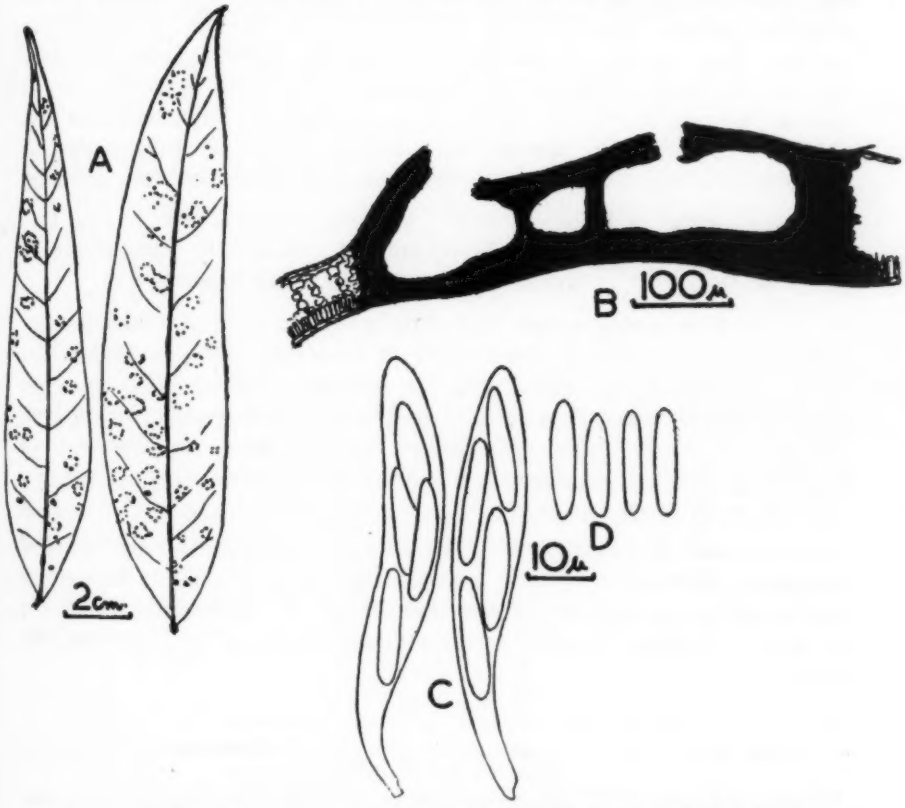

FIG. 4. *Phyllachara tetraspora*, A. Two leaves of *Polyalthia longifolia* showing the distribution of the stromata, B. Section through one stroma showing the loculi (diagrammatic), C. Asci, D. Ascospores.

central bare area, black, shining, circular, oval, elliptical or irregular in outline, 1–2 mm. in diam., slightly raised over the surface of the leaf, occupying the entire mesophyll of the leaf; loculi 1–3 in a stroma; asci fusiform to club-shaped, hyaline, 4 spored, $81–88 \times 11 \cdot 2\,\mu$; ascospores hyaline, long-elliptical

or oblong, sometimes slightly asymmetrical, arranged irregularly in two rows inside the ascus; 19–27×4·8 μ; paraphyses profuse, simple, hyaline.

On living leaves of *Polyalthia longifolia* Hk.f. and Th., Government Gardens, Trivandrum (T. C. State), 18–10–1952, coll. K. Ramakrishnan, [Herb. M.U.B.L. No. 882 (Type)].

Foliicola; maculæ nullæ; stromata visibilia in utroque latere foliorum, dispersa, vel aggregata bina vel terna, vel disposita in circulos circum aream centralem nudum, nigra, nitentia, circularia, ovalia, elliptica vel irregularia, 1–2 mm. diam., tenuiter elevata super folii paginam, implentia totam densitem mesophylli; loculi 1–3 in singulis stromatibus; asci fusiformes vel clavati, hyalini, 4-spori, 81–88×11·2 μ; ascosporæ hyalinæ, longe ellipticæ vel oblongæ, nonnumquam tenuiter asymmetricæ, dispositæ irregulariter in duplici serie in asco; 19–27×4·8 μ; paraphyses profusæ, simplices, hyalinæ.

Typus lectus in foliis viventibus *Polyalthiæ longifoliæ* Hk. f. and Th., Government Gardens, in loco Trivandrum (T.C. State) a K. Ramakrishnan, die 18 octobris 1952, et positus in Herb. M.U.B.L. sub numero 882.

Phyllachora maquilingensis Syd. has been described on *Polyalthia* sp. from Mount Maquiling in the Philippines (Saccardo, 1926, p. 566). This fungus has epiphyllous, black irregularly disposed stromata 250 μ in diam. The asci are cylindrical, 8 spored, 80×10–14 μ and the ascospores are hyaline, ellipsoid and 11–14×6–7 μ. My fungus differs from *P. maquilingensis* in having very much larger stromata which are amphigenous, four spored asci and very much larger ascospores.

I am grateful to Prof. T. S. Sadasivan for the material of *Polyclypeolum salvadoræ* which he very kindly placed at my disposal and for his critical reading of the manuscript. I also thank Prof. H. Santapau, S.J., of the St. Xavier's College, Bombay, for very kindly rendering the diagnoses into Latin.

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CHEMICAL CHANGES DURING FLUE-CURING AND THEIR BEARING ON THE QUALITY OF LEAF OF CIGARETTE TOBACCO*

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Introduction

The flue-curing of tobacco is an important step in the production of cigarette type of leaf and the success of curing determines to a great extent the quality and value of leaf produced. The techniques evolved for this process are more or less empirical and a thorough study of the chemical processes occurring in the leaf during curing may suggest ways of improving it. The work of Ward (1942) is the most relevant to the present investigation but he has determined only the changes in the carbohydrate fractions during flue-curing and the changes in the nitrogenous constituents are not known. The work of Askew and co-workers (Askew and Blick, 1947; Askew, 1947 and Askew, 1948) which has been reported after the present investigations have been completed, includes the changes in proteins also, besides carbohydrates, but the changes in the other nitrogenous fractions like ammonia and amides have not been determined. Studies reported in the following pages have therefore been undertaken and they include (1) chemical changes that occur during the flue-curing process and (2) the effect of such changes on the quality of leaf produced.

Experimental

Material and Methods.—Harrison Special, a well-established flue-cured variety of tobacco in India, has been employed in this investigation.

In all, four curings were studied, and for convenience in reference, they will be termed hereafter as curings A, B, C and D. The details of the leaf employed in these curings are given below:

1. *Curing A.*—2nd priming leaves from the 'No-manure' plots of the manurial experiment, Tobacco Research Station, Guntur, 1944.

* The Master of Science degree of Bombay University has been awarded for this work in 1946.

2. *Curing B.*—3rd priming leaves from the ' No-manure ' plots of the manurial experiment, Tobacco Research Station, Guntur, 1944.

3. *Curing C.*—6th priming leaves from the ' No-manure ' plots of the manurial experiment, Tobacco Research Station, Guntur, 1945.

4. *Curing D.*—7th priming leaves from the ' No-manure ' plots of the manurial experiment, Tobacco Research Station, Guntur, 1945.

The leaves were primed from the plant at the right stage of maturity, as judged by the light-tinted flecks so characteristic of ripe leaf (Garner, 1909).

Sampling.—The leaf was divided into the required number of lots by random sampling. Their green weight was determined and they were strung on separate sticks which were suitably labelled and removed from the barn during curing at the times noted in Tables I, II, III and IV. Leaf was immediately weighed and preserved as indicated below. The difference between the initial weight and this weight gave the loss in weight during curing upto the time of removal of the sample from the barn.

In curings A and B due to lack of material seven samples of 20 leaves each were collected. In curings C and D on the other hand, the number of samples taken was reduced and consequently samples of 100 leaves each could be used. Due to these reasons, sampling errors in curings A and B may be more than in C and D.

Preservation of samples.—In curings A and B, where the samples were small, the samples were preserved in alcohol (A.O.A.C., 1940). In curings C and D, where larger samples were dealt with, the samples were preserved by steaming and drying at 65° C. (Loomis and Shull, 1937).

At the time of analysis, the alcohol in the samples of curings A and B was removed under vacuum and they were dried at 65° C. and a correction applied for the calcium carbonate added at the time of preservation.

A description of the methods of analysis employed is given in the Appendix.

Expression of data.—The data in the present study are expressed in terms of 100 grams of the initial weight of leaves.

Conditions of curing in the present study.—The conditions of curing in these experiments are presented in Figs. 1 and 2.

It is clear from the data in Figs. 1 and 2 that conditions during yellowing (45 to 55 hrs.) in curings A, C and D were almost similar, while curing B was characterised by higher temperature and lower humidity.

PART I

CHEMICAL CHANGES IN THE LEAF DURING FLUE-CURING

The data representing the change in chemical constituents during flue-curing are presented in Tables I, II, III and IV and the changes under each individual item are discussed below.

WATER AND SOLIDS

By far the most important change in the composition of leaf during flue-curing is the loss in weight of leaves. This loss is the sum of the loss due to water and of solids during curing. The loss due to solids in these curings

TABLE I

Chemical changes during curing A *expressed as grams per* 100 *grams fresh leaf*

	Time in hours						
		Yellowing		Fixing		Drying	
	0	29	54	65	76	102	128
1. Loss in weight	0	29·37	31·53	46·48	53·39	78·43	80·01
2. Loss due to water ..	0	27·81	30·44	45·39	51·26	75·84	77·41
3. Total loss—loss due to water ..	0	1·56	1·09	1·09	2·13	2·59	2·60
4. Total solids ..	21·74	20·18	20·65	20·65	19·61	19·15	19·14
5. Inorganic solids	3·39	3·30	3·66	3·59	3·69	3·34	3·91
6. Organic solids ..	18·35	16·88	16·99	17·06	15·92	15·81	15·23
7. Starch as glucose	5·70	2·55	1·38	0·84	0·50	0·36	0·31
8. Reducing sugars as glucose ..	0·43	1·14	2·10	2·31	2·34	1·54	1·39
9. Sucrose as invert sugar ..	0	0	0·11	0·06	0·07	1·47	1·39
10. Total sugars (8+9) ..	0·43	1·14	2·21	2·37	2·41	3·01	2·78
11. Total carbohydrates (7+10)	6·13	3·69	3·59	3·21	2·91	3·37	3·09
12. Total N ..	0·364	0·363	0·358	0·375	0·362	0·345	0·372
13. Soluble N ..	0·144	0·246	0·273	0·304	0·288	0·282	0·289
14. Insoluble N ..	0·220	0·107	0·085	0·071	0·074	0·063	0·083
15. Nicotine N ..	0·043	0·039	0·039	0·043	0·043	0·043	0·045
16. Ammonia N ..	0·0027	0·0024	0·0025	0·0039	0·0030	0·0060	0·0069
17. Amide N×2 ..	0·018	0·016	0·016	0·026	0·029	0·026	0·032
18. Rest N ..	0·080	0·189	0·215	0·231	0·213	0·207	0·205
19. Phosphates (P_2O_5) ..	0·058						

TABLE II

Chemical changes during curing B *expressed as grams per* 100 *grams fresh leaf*

	Time in hours						
		Yellowing		Fixing		Drying	
	0	31	55	62	71	91	132
1. Loss in weight	0	15·08	24·39	28·63	44·06	65·00	80·71
2. Loss due to water	0	14·12	22·83	26·56	41·19	61·45	77·46
3. Total loss−loss due to water	0	0·96	1·56	2·07	2·87	3·55	3·25
4. Total solids ..	22·23	21·27	20·67	20·16	19·36	18·68	18·98
5. Inorganic solids	3·56	3·84	3·73	3·75	3·64	3·71	3·75
6. Organic solids ..	18·67	17·43	16·94	16·41	15·72	14·97	15·23
7. Starch as glucose	4·68	1·69	0·90	0·91	0·48	0·43	0·31
8. Reducing sugars as glucose ..	0·45	2·05	2·67	2·63	2·69	2·39	1·46
9. Sucrose as invert sugar ..	0	0	0·08	0·12	0·16	0·52	1·31
10. Total sugars (8+9) ..	0·45	2·05	2·75	2·75	2·85	2·91	2·77
11. Total carbohydrates (7+10)	5·13	3·74	3·65	3·66	3·33	3·34	3·08
12. Total N ..	0·434	0·427	0·413	0·445	0·424	0·426	0·427
13. Soluble N ..	0·211	0·252	0·248	0·290	0·279	0·269	0·283
14. Insoluble N ..	0·223	0·175	0·165	0·155	0·145	0·157	0·144
15. Nicotine N ..	0·052	0·052	0·049	0·051	0·051	0·054	0·052
16. Ammonia N ..	0·003	0·003	0·004	0·004	0·005	0·005	0·009
17. Amide N×2 ..	0·020	0·016	0·021	0·026	0·023	0·024	0·027
18. Rest N ..	0·136	0·181	0·174	0·209	0·200	0·186	0·195
19. Phosphates (P_2O_5) ..	0·054						

ranges from 2·5% to 3·25%, which is 12 to 16% of the solids originally present in the leaf. A major portion of the loss in weight is due to water which ranges from 77·41% to 79·54%, nearly four-fifths to the initial weight of leaves.

The rates of loss of water are shown by the slopes of the curves in Fig. 3. From these it appears that in curings C and D the rate of loss is more than in curings A and B. Petrenko's (1940) observation that leaf possessing more carbohydrates lost its moisture more slowly because of the formation of hydrophilic colloids may explain this. But this is not enough to explain the loss of moisture in curing B which is slower than in A. The leaf in curing B

TABLE III

Chemical changes during curing C *expressed as grams per* 100 *grams fresh leaf*

		Time in hours			
			Yellowing	Fixing	Drying
		0	46	74	151
1.	Loss in weight	0	30·53	53·48	82·47
2.	Loss due to water	0	28·94	51·10	79·96
3.	Total loss—loss due to water ..	0	1·59	2·38	2·51
4.	Total solids	20·04	18·45	17·66	17·53
5.	Inorganic solids	2·64	2·64	2·68	2·59
6.	Organic solids	17·40	15·81	14·98	14·94
7.	Soluble solids	6·33	8·44	8·56	8·81
8.	Insoluble solids	13·71	10·01	9·10	8·72
9.	Soluble inorganic solids ..	1·60	1·65	1·72	1·60
10.	Soluble organic solids ..	4·73	6·79	6·84	7·21
11.	Insoluble organic solids ..	12·67	9·02	8·14	7·73
12.	Ether extract	1·42	1·24	1·21	1·25
13.	Starch as glucose	4·15	1·04	0·56	0·27
14.	Reducing sugars as glucose ..	0·54	1·56	1·51	0·96
15.	Sucrose as invert sugar ..	0·04	1·02	1·07	1·72
16.	Total sugars (14+15)	0·58	2·58	2·58	2·68
17.	Total carbohydrates (13+16) ..	4·73	3·62	3·14	2·95
18.	Total N	0·345	0·341	0·337	0·334
19.	Soluble N	0·155	0·185	0·201	0·200
20.	Insoluble N	0·190	0·156	0·136	0·134
21.	Nicotine N	0·038	0·034	0·040	0·034
22.	Ammonia N	0·004	0·004	0·006	0·005
23.	Amide N×2	0·021	0·048	0·039	0·039
24.	Rest N	0·092	0·099	0·116	0·122
25.	Phosphates (P_2O_5)	0·119			

contained less carbohydrates than that in curing A, and also higher temperature and lower humidity prevailed during its yellowing. Both these factors should have caused more rapid loss of water in curing B than in A. But the reverse happened. This may be because water in leaves of curing A (2nd primings) was more loosely held than in those of curing B (3rd primings). The observation of Ward (1942) that in the first and second primings water is more loosely held than in third primings probably explains this.

Inorganic solids.—Inorganic solids remain unchanged during flue-curing and the loss in solids is entirely borne by the organic solids.

TABLE IV

Chemical changes during curing D *expressed as grams per* 100 *grams fresh leaf*

		Time in hours			
			Yellowing	Fixing	Drying
		0	54	71	151
1.	Loss in weight	0	40·01	62·50	82·36
2.	Loss due to water	0	38·28	60·49	79·54
3.	Total loss − loss due to water ..	0	1·73	2·01	2·82
4.	Total solids	20·46	18·73	18·45	17·64
5.	Inorganic solids	2·75	2·75	2·79	2·79
6.	Organic solids	17·71	15·98	15·68	14·85
7.	Soluble solids	6·63	8·18	8·03	8·09
8.	Insoluble solids	13·83	10·55	10·42	9·55
9.	Soluble inorganic solids ..	1·56	1·52	1·52	1·51
10.	Soluble organic solids ..	5·07	6·66	6·51	6·58
11.	Insoluble organic solids ..	12·64	9·32	9·15	8·27
12.	Ether extract	1·63	1·46	1·46	1·41
13.	Starch as glucose	2·94	0·29	0·20	0·19
14.	Reducing sugars as glucose ..	0·51	1·44	1·17	0·63
15.	Sucrose as invert sugar ..	0	0·62	0·79	0·96
16.	Total sugars (14+15)	0·51	2·06	1·96	1·59
17.	Total carbohydrates (13+16)	3·45	2·35	2·16	1·78
18.	Total N	0·423	0·423	0·411	0·403
19.	Soluble N	0·131	0·252	0·256	0·271
20.	Insoluble N	0·292	0·171	0·155	0·132
21.	Nicotine N	0·039	0·040	0·043	0·035
22.	Ammonia N	0·003	0·008	0·008	0·007
23.	Amide N×2	0·034	0·09	0·096	0·094
24.	Rest N	0·055	0·114	0·109	0·135
25.	Phosphates (P_2O_5)	0·127			

Insoluble and soluble solids.—In view of the smaller sampling errors that were possible in the samples of curings C and D, soluble solids and ether extractable materials also were determined in these samples.

Insoluble solids decreased sharply during yellowing and more slowly during the later stages of curing. As a result of this soluble solids increased considerably during yellowing and thereafter the increase was very slow. The increase in soluble solids is due to the hydrolysis of starch and proteins.

Ether-soluble substances.—The contribution of ether-soluble substances to the loss in organic solids is only 0·2 gram in both the curings and most of it occurs during yellowing.

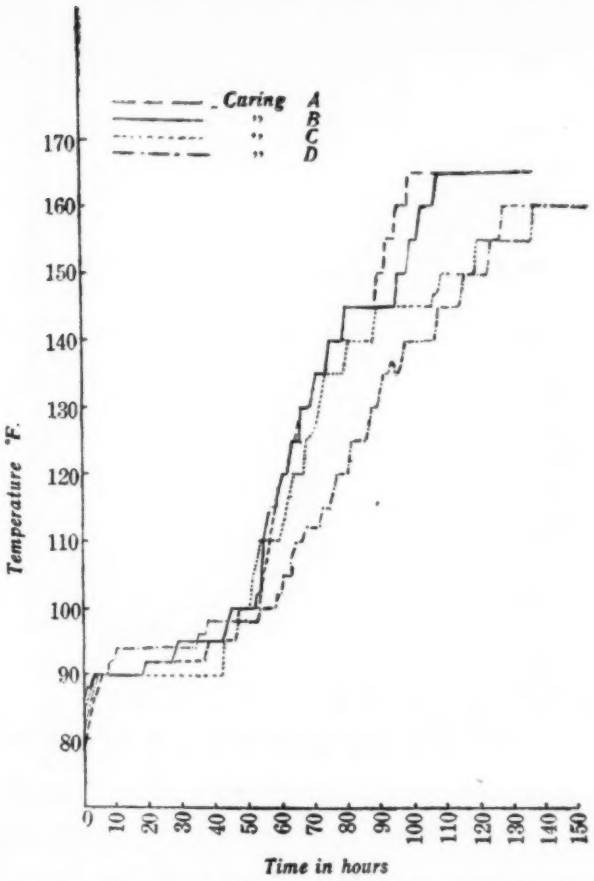

FIG. 1. Temperature in the barn during Flue-curing of tobacco.

THE CARBOHYDRATE FRACTIONS

Starch.—In all these leaves starch is the chief reserve carbohydrate and the most striking phenomenon is its hydrolysis and disappearance during yellowing. Expressed in terms of the quantity originally present in the leaf this comes to 75·78% in curing A, 80·78% in B, 74·95% in C and 90·14% in D.

The hydrolysis of starch progresses though at a much reduced rate throughout the fixing period, and during drying the changes in this constituent are insignificant.

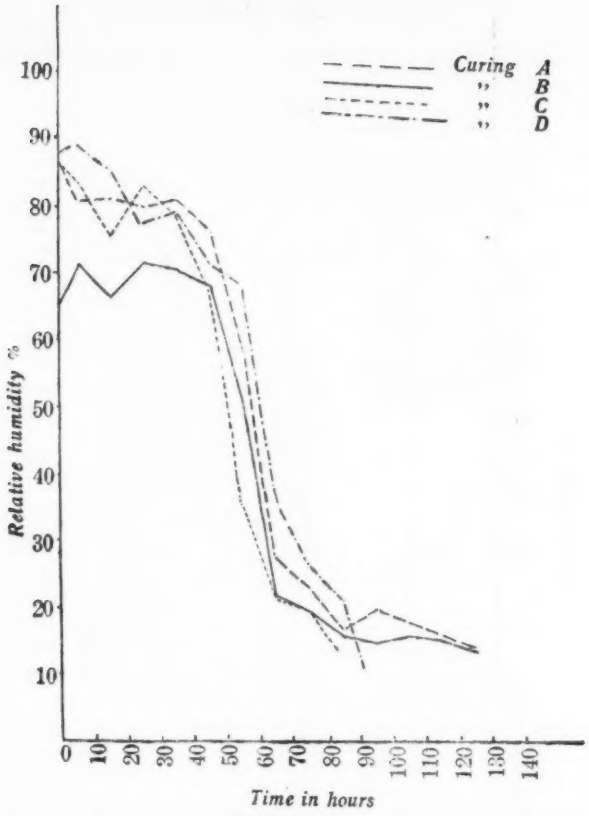

FIG. 2. Relative Humidity in the barn during Flue-curing of tobacco.

Reducing sugars.—As starch hydrolysis progresses, reducing sugars increase enormously during yellowing, remain steady during "fixing" and decrease rapidly during drying. They are mostly glucose and fructose, as maltose could not be detected at any stage of the curing process (The familiar osazone test was used for the detection of maltose). The break-down of starch must have been extremely rapid as the intermediate maltose could not be detected. Examination of Ward's results (1942) shows that levulose and glucose reach maxima and then decrease while sucrose continues to increase till the end of curing. Askew and Blick (1947) on the other hand could not detect any such maxima for these sugars. The present studies show that reducing sugars reach a maximum about the end of yellowing, while sucrose continues to increase till the end of curing.

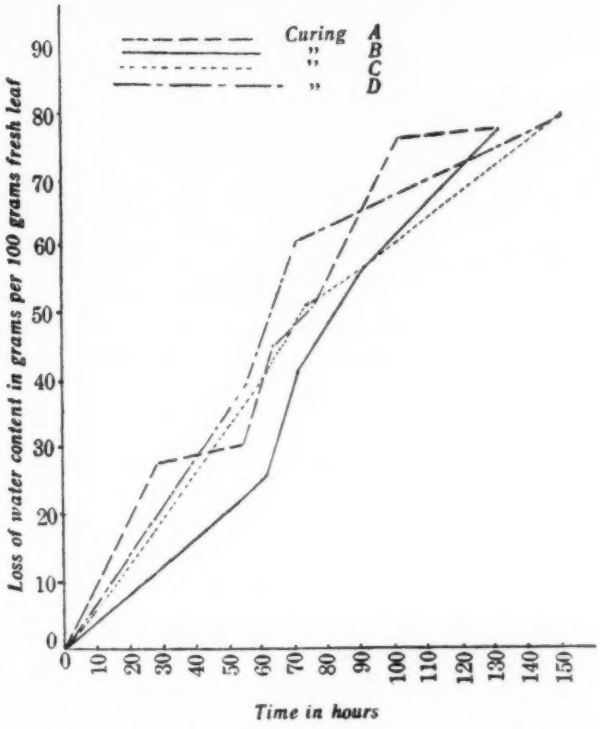

FIG. 3. Changes in the water content of the leaf during Flue-curing of tobacco.

But the increase in sugars during yellowing is not enough to account for the loss in starch. Ward (1942), on the other hand, found that the loss in starch could not fully account for the high percentages of sugars and stated that probably glucosides also contributed to sugar formation. Canadian leaves probably contain a higher percentage of glucosides as compared with Indian leaves.

Sucrose.—Sucrose increases during flue-curing from almost nothing to about 1½ grams. But there are differences in its formation in the four sets of leaves studied. In curings A and B no appreciable quantities of this sugar were formed during yellowing, while in curings C and D, 1·02 grams and 0·62 gram were formed during yellowing alone. During the " fixing " stage no appreciable quantity of sucrose was formed in any of the curings, while a considerable amount of sucrose was synthesised in all the curings during the drying period.

A reference to literature suggests two factors which appear to influence sucrose synthesis in leaves. One is desiccation of the tissues (Hart, 1937; Leonard, 1938 and 1939; Schroeder *et al.*, 1922, 1931; Nelson *et al.*, 1933) and the other is phosphorylation (Burkhard, 1934; Tanko, 1936; Hassid, 1938; Kursanov *et al.*, 1939; and Hart, 1943, 1944).

Viewed in the light of these two observations the differences in sucrose formation in these leaves become explicable. The phosphate content of the leaves in curings C and D was double that of leaves in curings A and B. The desiccation of tissues during yellowing was not enough for sucrose synthesis because of the low level of phosphates in the leaves of curings A and B; while considerable amounts of sucrose were produced during yellowing in the leaves of curings C and D, because of their high phosphate content.

NITROGENOUS FRACTIONS

Total nitrogen.—There is no significant change in the total nitrogen of the leaf during the flue-curing process.

Protein nitrogen.—The nitrogenous fraction which is insoluble in water, and which may be taken as protein, decreases rapidly in all the curings during yellowing but later on the loss is much less rapid.

The loss in insoluble N during yellowing expressed as a percentage of the total loss during the entire curing process, ranges from 98% in curing A to 76% in curing D. Thus in all the curings, most of the hydrolysis of proteins occurs during yellowing only.

Soluble N.—As hydrolysis of proteins in the leaves proceeds the soluble nitrogen increases and this increase is the greatest during yellowing.

Nicotine N.—While the change in nicotine content during curings A and B is not significant, about 4 milligrams of nicotine nitrogen appears to have been lost during curings C and D. Slight decreases in the nicotine content of the leaves, amounting to 3 to 5% of that originally present were recorded by many workers (Bernardini, 1922; Haley, 1944; Smirnov *et al.*, 1930; Vickery *et al.*, 1931).

Ammonia and amide nitrogen.—The data indicate that in curings A, B and C there was no significant increase in ammonia N during yellowing; while during fixing and drying this increased. In curing D on the other hand ammonia increased by nearly three times during yellowing itself and thereafter there was no increase.

As regards amide N also, there were differences in their distribution in the four curings under study. In curings A and B, there was no increase in

amide N during yellowing, while in curings C and D amide nitrogen reached a maximum at the end of yellowing, being almost double that originally present in the leaf.

Discussion

Data presented previously show that besides desiccation of tissues, the other important chemical changes during flue-curing include hydrolysis and solubilization of starch and proteins. As a result of this nearly 30 to 40% of the insoluble solids are converted into soluble forms. The contribution of the various insoluble fractions to this conversion is indicated in Table V below.

Table V

(Results expressed in 100 grams fresh leaf)

	Curing C	Curing D
Loss in insoluble solids	4·94	4·37
Loss in protein (Insoluble N × 6·25)	0·35	1·00
Loss in starch	3·49	2·48
Loss in starch % / Loss in insoluble solids ..	70·65	56·75
Loss in protein % / Loss in insoluble solids ..	7·10	22·90

The above data show that in flue-curing changes due to hydrolysis of starch appear to be of far greater moment than changes due to degradation and hydrolysis of proteins. This is in sharp contrast to what happens in the Connecticut shade-grown tobacco, a cigar type, in which Vickery *et al.* (1931) found that 70% of the loss of insoluble solids is due to hydrolysis of proteins.

From the foregoing it appears that in flue-curing changes due to transformations of carbohydrates are of far greater moment than those due to nitrogenous constituents. But the degree to which the various constituents take part in these transformations appears to depend on the initial chemical composition of the leaves. Leaves in curing A are the richest in carbohydrates while leaves in curing D are the poorest. Leaves in B and C have intermediate composition. This appears to determine the path of chemical

reactions as well as the quality produced. In Table VI are given the losses in organic solids and carbohydrates during the various stages of curing.

TABLE VI

	Yellowing		Fixation of colour		Drying	
	Organic solids	Carbo-hydrate	Organic solids	Carbo-hydrate	Organic solids	Carbo-hydrate
Curing A ..	−1·36	−2·54	−1·07	−0·68	−0·69	−0·18
Curing B ..	−1·73	−1·48	−1·22	−0·32	−0·49	−0·25
Curing C ..	−1·60	−1·11	−0·83	−0·48	−0·04	−0·15
Curing D ..	−1·73	−1·10	−0·32	−0·19	−0·81	−0·38

It is obvious from the data in the above table that during yellowing which is a life-process, carbohydrates more than account for the loss in organic solids in curing A, while in curing D they contribute only towards 64% of this loss. In the later case, probably other substances were drawn in for respiratory purposes.

The differences in ammonia and amide formation in the four curings under study, appear to point out to this possibility. In curings A and B where the initial level of starch was high, no ammonia or amides were formed during yellowing, while in curing C though there was no change in ammonia, amide N was more than doubled at the end of yellowing, while in curing D both ammonia and amide increased by about three times by the end of yellowing.

Literature appears to suggest two factors to be responsible for the formation of amides in plant tissues. They are (1) the concentration of ammonia and (2) the *a*-keto acids which are the precursors of the amides (Vickery *et al.*, 1939 *a* and 1939 *b*; Wood and Petrie, 1938; Wood, 1942; and Mothes, 1933).

Viewed in this light the differences in ammonia and amide formation in the four curings under study become explicable. The yellowing process in flue-curing which is essentially a life-process is hastened and shortened—it took only 48 to 55 hours in these studies while in the air-curing operations reported by Vickery *et al.* (1931) it took 12 days and in the culture experiments

in the dark of Vickery *et al.* (1937) it took 143 hours for the leaves to yellow—and the chemical changes that occur during yellowing are due to the hastening of the katabolism of the starving leaf. In such a system, the leaf relies solely on carbohydrates, for carbohydrates appear to be the most easily available substrates for respiration. In curings A and B where the initial starch content was high, the carbohydrates were enough to supply all the energy required for respiration and there was no necessity to draw on amino-acids for this purpose, oxidative deamination of amino-acids and accumulation ammonia and amides during yellowing being thus not important. In curing C, the leaf contained smaller amounts of carbohydrates than in curings A and B. Amino-acids had to be used for respiration, but there was no accumulation of ammonia, as all the ammonia was perhaps used in amide synthesis, because there was no dearth of the necessary precursors, the *a*-keto acids.

In curing D, on the other hand, the leaves contained the smallest amount of carbohydrates and amino-acids were drawn on for respiratory purposes. Both ammonia and amides accumulated during yellowing, probably because there was dearth of *a*-keto acids which are of carbohydrate origin, and all the accumulated ammonia could not be used in amide formation.

Thus all the chemical changes observed in the leaf during flue-curing appear to depend on the initial carbohydrate level of the leaf. This is reflected in the quality of leaf produced also and this has been dealt with in Part II.

PART II

The Effect of the Chemical Changes during Flue-Curing on the Quality of Leaf Produced

A detailed study of the chemical changes that occur during flue-curing has been reported in Part I. It is necessary to see the relationship of these changes to the quality of leaf produced, for quality of the leaf is, as it were, the ultimate test of the efficiency of the curing process.

For this purpose, the leaves obtained in the different curings were graded according to commercial standards and the data presented in Table VII.

From the above data it is clear that curing A undoubtedly produced more of the best quality leaf and next in order were curings B, C and D respectively. The large quantities of green grades produced in curings C and D are also worthy of note.

TABLE VII

Percentage of each grade of leaf obtained during the curings A, B, C and D

Grade	Curing A	Curing B	Curing C	Curing D
1 ..	..	..	..	..
2 ..	7·69	1·96	..	..
3 ..	34·62	42·65	38·98	7·49
4 ..	41·34	49·21	16·52	29·96
5 ..	14·42	12·74	5·51	31·46
Light green ..	..	1·47	11·02	..
Dark green ..	0·96	0·49	26·27	28·46
Scrap ..	0·96	0·47	1·69	2·62

Several workers tried to correlate quality of the cured leaf with its chemical composition and introduced

(1) the ratio of carbohydrate to protein (Schmuck, 1924);

(2) the ratio of carbohydrates to polyphenol (Schmuck, 1927);

(3) the ratio of carbohydrates to total nitrogen excluding nicotine N (Kavalenko, 1935);

(4) the carbohydrates alone (Darkis *et al.*, 1936)

as indices of quality, but some at least realised that there were some abnormal tobaccos in which these ratios were not associated with quality (Darkis *et al.*, 1936; and Schmuck, 1927).

In Table VIII are given the various ratios of chemical constituents in the leaf obtained during the four curings under investigation.

When it is noted that the quality of cured leaf decreases in the curings A, B, C and D in that order, it will be seen that none of the above ratios correctly represents the quality of the leaf. However, it is completely explained when the initial starch content of the green leaf is taken into consideration. As the starch content of the green leaf falls, the quality of the cured leaf worsens.

Thus the whole question of the quality of leaf produced during flue-curing, where the bright lemon yellow colour of the leaf is of paramount importance, appears to revolve round the carbohydrate katabolism of the leaf during yellowing.

TABLE VIII

Ratios of chemical constituents associated with quality in the cured leaf obtained in the curings A, B, C and D

	Reducing sugars	Total sugars	Total sugars ÷Protein N	Total sugars ÷(Total N —Nicotine N)	Starch in the green leaf %
	1	2	3	4	5
Curing A ..	7·26	14·52	33·77	8·52	5·70
Curing B ..	7·70	14·58	19·19	7·38	4·68
Curing C ..	5·46	15·25	20·06	8·45	4·15
Curing D ..	3·55	8·98	12·13	4·31	2·94

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SUMMARY

1. During flue-curing, as in air-curing, extensive desiccation of the leaf tissue occurred. The fresh leaf contained nearly 80% of its weight of water and most of it was lost during curing.

2. Total solids amounting to nearly 15% of the solids, originally present in the leaf, were lost during flue-curing. As the inorganic solids remained constant, almost the whole of this loss was borne by organic solids.

3. Extensive hydrolysis of starch, proteins and other constituents, probably of carbohydrate origin, occurred. The contribution of starch to this process was 60 to 70% while proteins contributed only 7 to 20%. Thus in flue-curing changes due to hydrolysis of starch are of far greater moment than changes due to degradation and hydrolysis of proteins and this is just the opposite of what was reported for cigar tobacco.

4. 0·2 gram of ether-soluble substances were lost mostly during yellowing.

5. Reducing sugars consisting mostly of glucose and fructose, increased at the expense of starch but this increase was not enough to account for the loss in starch. This was due to respiratory losses.

6. About 1½ grams of sucrose were formed during flue-curing. Differences in its formation in the four curings studied were found to be due to differences in the phosphate content of leaves.

7. Changes in the nitrogenous constituents indicated that much of the leaf protein underwent hydrolysis into soluble forms. Differences in the formation of amides and ammonia in the four curings were found to be due to differences in the availability of carbohydrates for respiratory purposes.

8. Studies on the quality of the leaf produced in these curings indicated that the quality of leaf was dependent on the initial starch content of the leaf.

9. Thus all the reactions in the leaf during the flue-curing process and the quality of the leaf appear to centre round the carbohydrate content of the leaves.

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APPENDIX

Method of Analysis

1. *Total solids.*—The samples dried at 65° C. are powdered and moisture on a suitable aliquot of the sample determined, so that the results may be brought to dry basis and the initial green weight basis.

2. *Moisture.*—Determined by drying 2 gr. of the powdered sample at 100° C. for 3 hours, cooling in a desiccator and weighing (Woodmansee, Rapp and McHargue, 1942).

3. *Total soluble solids.*—2 gr. of the sample is weighed into a beaker, treated with 25 c.c. of water, and kept on the water-bath for 15 minutes. The supernatant liquid is decanted on to a filter paper, and the residue in the beaker is washed with hot water, till all the soluble solids are extracted. The filtrate is made upto 100 c.c. preserved with a few drops of toluene and kept in the frigidaire for the determination of soluble nitrogen, ammonia and amides. A 50 c.c. aliquot of this extract is evaporated to dryness in platinum dishes and dried to constant weight at 105° C.

4. *Inorganic solids.*—A known weight of the sample is ignited over a bunsen burner fitted with a rose-head, till ash of constant weight is obtained.

5. *Soluble inorganic solids.*—The solids from the soluble solids determination (3) are ignited over a bunsen burner fitted with rose-head, till ash of constant weight is obtained.

6. *Total organic solids.*—Calculated by difference between total solids (1) and ash (4).

7. *Soluble organic solids.*—Calculated by difference between (3) and (5).

8. *Total nitrogen.*—Determined by the method described by Tow and Earl (1940).

9. *Soluble Nitrogen.*—A suitable aliquot of the water extract (3) is taken into a kjeldahl flask and is evaporated to dryness by immersing in a water-bath and connecting to a vacuum pump. After all the moisture is removed, the nitrogen is determined as in (8).

10. *Nicotine.*—Determined by Worseley's method (1927).

11. *Ammonia.*—Determined by the method of A.O.A.C. (1940) on a suitable aliquot of the water extract (3).

12. *Amide nitrogen.*—Determined on a suitable aliquot of the water extract (3) by the method of Vickery and Pucher (1931 *a*).

13. *Reducing sugars.*—0·5 to 1·0 gram of the powdered sample is extracted with 80% alcohol and the alcoholic extract is made to volume. The residue is saved for the determination of starch. A suitable aliquot of the extract is taken in a beaker, the alcohol removed by evaporation on a water-bath, small quantities of water being added occasionally, to avoid evaporation to complete dryness. It is now transferred to a volumetric flask, 1 c.c. of saturated neutral lead acetate solution is added and made to volume. After shaking thoroughly, it is filtered through a dry filter paper and funnel, deleaded by adding a pinch of powdered sodium oxalate, filtered again on a dry paper, and 5 c.c. of this is taken for estimation of reducing sugars, which are determined by the method described by Van Der Plank (1936).

14. *Sucrose.*—25 c.c. of the deleaded filtrate from the above (13) is inverted by adding hydrochloric acid according to A.O.A.C. (1940). The acid is neutralised after inversion, with sodium carbonate and the volume is made upto 50 c.c. 5 c.c. aliquot of this is used for the estimation of reducing sugars, which is carried according to Van Der Plank's method (1936). The increase in reducing sugars after inversion is taken as sucrose.

15. *Starch.*—The residue after alcoholic extraction (13) is dried and starch determined according to the method described by Loomis and Shull (1937, p. 286).

16. *Ether Extract.*—Determined by the method of A.O.A.C. (1940, p. 356).

H-ION CONCENTRATION IN THE ALIMENTARY CANAL OF TWO SPECIES OF TELEOSTS, *COTTUS SCORPIUS* AND *ZOARCES VIVIPARUS*

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Introduction

Among the different classes of Pisces, the Teleostei includes the largest number of species and display the greatest diversity of feeding habits. Thus Teleostean forms have stimulated many workers to the study of the alimentary canal, but despite the voluminous work in the field, comparatively little attention has been paid to its physiology.

In the study of H-ion concentration in the alimentary canal of Pisces, various research workers (for example, Herwerden, 1908; Vonk, 1927) have found that the stomach of Teleosts is less acidic than that of Selachians. The pH values of the stomach of teleosts have, so far, been observed to be generally above 3. Bayliss (1935) is the only author who records pH values of 2·4 and 2·7 in the stomach of two specimens of *Pleuronectes platessa*, although the pH values in the stomach of 23 others varied between 5·5 and 7·6. Determining the reactions in the stomach by Congo paper, Kenyon (1925) found that the pH values in the stomach of several teleosts varied from neutrality to pH 4. Vonk (1927) found that the pH in the stomach of *Esox lucius* varied from 4·92 to 5·97. Karpevitch and Bokaff (1937) found in *Pleuronectes flessus* that the pH in the stomach, after the fish had been fed, was, at first, 7·4, but fell to 5·5 towards the end of digestion.

The object of the present work was to determine the H-ion concentration in the alimentary canal of the teleosts with the help of more modern apparatus by using pH electric potentiometer and to correlate the results with the histological structures. *Cottus scorpius* (L.) and *Zoarces viviparus* (L.) were the two species selected for the course of this work.

Materials and Methods

All specimens used in this work were obtained from Marine Biological Station, Den Helder (Holland), and were kept in the aquarium tanks at the

Laboratory of Comparative Physiology, University of Utrecht, Utrecht (Holland).

The pH measurements were taken in starving and artificially fed fish. The starved fish were kept in clean glass aquarium tanks for 7 days without any food. After this period, food was introduced into the stomach by pushing it gently with a glass rod from the buccal cavity into the stomach. The pH measurements were taken after the food had been in the stomach for 24, 48 and 72 hours.

The fish were killed by pithing, and this operation as well as the removal of the alimentary canal had to be carried out with great care in order to avoid pressing the duodenal fluid into the stomach or *vice-versa* before the stomach was ligatured at either end. The stomach was then brushed with distilled water to wash away the body fluids from its outer wall in order to determine more correctly the pH of the fluid when it was cut open.

The pH measurements were taken on a Cambridge potentiometer, using quinhydrone electrode.

Observations

The following pH measurements were taken at different locations of the alimentary canal of *Cottus scorpius* and *Zoarces viviparus*.

(*a*) *Cottus scorpius*

Fish No. 1.—A specimen from an aquarium tank was taken, but no observations were made on its feeding. The stomach was empty. Very little food was found in the posterior part of the intestine (Fig. 1 *a*).

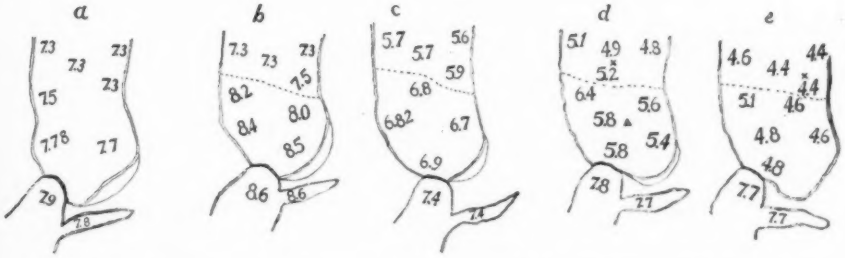

FIG. 1. pH measurments in the stomach, duodenum and pyloric cæcum of *Cottus scorpius*. The anterior and posterior regions of the stomach are divided by a dotted line. (*a*) Random specimen, (*b*) Starved specimen, (*c*) 24 hours after giving food, (*d*) 48 hours after giving food, (*e*) 72 hours after giving food.

LOCATION	pH
(1) Fluid oozing from the middle region of the stomach when it was cut open	7·7
(2) Four different places on the mucosal wall of the anterior portion of the stomach	7·3, 7·3, 7·3, 7·3
(3) At 3 different places on the mucosal wall of the posterior region of the stomach	7·5, 7·7 7·8
(4) In the duodenum	7·9
(5) In the pyloric cæcum	7·8

Fish No. 2.—A fish was kept without food for 7 days in an aquarium tank containing clear sea-water. No food was found in the alimentary canal after the stravation period (Fig. 1 *b*).

LOCATION	pH
(1) Fluid (very little) oozing from the middle region of the stomach when it was cut open	7·6
(2) At 4 different places in the anterior region of the stomach	7·3, 7·3 7·3, 7·5
(3) At 4 different places in the posterior region of the stomach	8·0, 8·2 8·4, 8·5
(4) In the duodenum	8·6
(5) In the pyloric cæcum	8·6

Fish No. 3.—The fish was fed with a meal of small *Mytilus edulis* with valves open, sliced fish, chopped flesh of *Mytilus edulis* and a crab. pH measurements were taken after the food had been in the stomach for 24 hours. No food had passed from the stomach into the intestine (Fig. 1 *c*).

LOCATION	pH
(1) Fluid oozing from the middle region of the stomach after it had been cut open	5·85
(2) On a piece of sliced fish	5·6
(3) At 4 different places in the anterior region of the stomach	5·6, 5·7, 5·7, 5·9
(4) At 4 different places in the posterior region of the stomach	6·7, 6·8, 6·82, 6·9
(5) In the duodenum	7·4
(6) In the pyloric cæcum	7·4

Fish No. 4.—A fish was fed on the same meal as was given to Fish No. 3. pH measurements were taken after the food had been in the stomach for 48 hours. No food had passed into the intestine (Fig. 1 *d*).

Location	pH
(1) Fluid oozing from the middle region of the stomach when it was cut open	4·4
(2) On the inside of opened *Mytilus edulis* after the stomach had been cut open	4·5
(3) Fluid lying in the depression of the mucosal wall after the removal of *Mytilus edulis*	4·08
(4) On a piece of sliced fish lying in the anterior region of the stomach (marked ×)..	3·6
(5) At 4 different places in the anterior region of the stomach	4·8, 4·9, 5·1, 5·2
(6) On a piece of the flesh of the crab lying in the posterior region of the stomach	4·3
(7) On a piece of sliced fish lying in the posterior region (marked Δ)	4·46
(8) At 5 different places in the posterior region of the stomach	5·4, 5·6 5·8, 5·8 6·4
(9) In the duodenum	7·8
(10) In the pyloric cæcum	7·7

Fish No. 5.—A fish was fed on the same meal as Fish No. 3. pH measurements were taken after the food had been in the stomach for 72 hours. A few slices of chopped fish had passed into the intestine (Fig. 1 *e*).

Location	pH
(1) Fluid oozing from the middle region of the stomach..	4·2
(2) On a soft piece of the flesh of the crab lying in the anterior region of the stomach (marked ×) ..	3·42
(3) At 4 different places in the anterior region of the stomach	4·4, 4·4, 4·4, 4·6
(4) On the inside of *Mytilus edulis*	4·3
(5) On the flesh of the crab lying in the posterior region of the stomach	4·5
(6) At 5 different places in the posterior region of the stomach	4·6, 4·6, 4·8, 4·8 5·1
(7) In the duodenum	7·7
(8) In the pyloric cæcum	7·7

(*b*) *Zoarces viviparus*

Fish No. 1.—A fish was kept for 7 days in an aquarium tank. The alimentary canal was found to be devoid of food after the starvation period (Fig. 2 *a*).

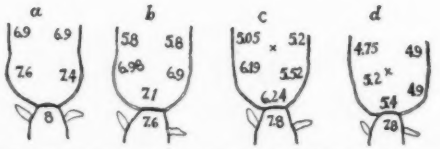

FIG. 2. pH measusements in the stomach and duodenum of *Z. viviparus*. (*a*) Starved specimen, (*b*) 24 hours after giving food, (*c*) 48 hours after giving food, (*d*) 72 hours after giving food.

LOCATION	pH
(1) Fluid oozing from the stomach	7·2
(2) At 2 different places in the anterior region of the stomach	6·9, 6·9
(3) At 2 different places in the posterior region of the stomach	7·6, 7·4
(4) In the duodenum	8·0

Fish No. 2.—A fish was fed on chopped crab and fish. pH measurements were taken after the food had been in the stomach for 24 hours. No food had passed into the intestine (Fig. 2 *b*).

LOCATION	pH
(1) Fluid oozing from the stomach	6·85
(2) On the flesh of the crab	6·64
(3) On a piece of fish	6·72
(4) At 2 different places in the anterior region of the stomach	5·8, 5·8
(5) At 3 different places in the posterior region of the stomach	6·9, 6·98 7·1
(6) In the duodenum	7·6

Fish No. 3.—A fish was fed on the same meal as fish No. 2. The food remained in the stomach for 48 hours without being passed into the intestine (Fig. 2 *c*).

LOCATION	pH
(1) Fluid oozing from the stomach	5·75
(2) On the flesh of the crab lying in the anterior region of the stomach (marked ×)	5·1
(3) At 2 different places in the anterior region of the stomach	5·05, 5·2
(4) At 3 different places in the posterior region of the stomach	5·52, 6·19 6·24
(5) In the duodenum	7·8

Fish No. 4.—A fish was fed with the same meal as Fish No. 2. The food remained in the stomach for 72 hours without being passed into the intestine (Fig. 2 *d*).

Location	pH
(1) Fluid oozing from the stomach	4·8
(2) On the flesh of fish lying in the middle region of the stomach (marked ×)	4·7
(3) On the flesh of the crab also lying in the middle region of the stomach	4·95
(4) At 2 different places in the anterior region of the stomach	4·75, 4·9
(5) At 3 different places in the posterior region of the stomach	4·9, 5·2 5·4
(6) In the duodenum	7·8

Discussion and Conclusion

The reactions in the stomachs of the starved specimens of *Cottus scorpius* and *Zoarces viviparus* might have been neutral or alkaline, but were not acid. The neutral or alkaline reactions in the stomach would suggest that either no acid was secreted or that the acid secreted was neutralised. It is not yet known whether secretion of acid in the stomach of any vertebrate is a continuous process or whether it is intermittent. If acid is secreted when no food is present in the stomach, it must be neutralised by either the alkaline gastric mucus or the regurgitation of alkaline intestinal fluids. The gastric mucus in mammals is alkaline and has an acid-binding property (Babkin, 1944, p. 214). Bayliss (1935) suggests the possibility of acid in the stomach of *Pleuronectes platessa* being neutralised by the intestinal fluid. In *C. scorpius* and *Z. viviparus* the possibility of gastric mucus neutralising the acid secretion during starvation is greater than that of intestinal regurgitation, as the opening between the stomach and the intestine is greatly constricted and is also provided with gastro-intestinal fold.

The gastric contents are acid while food is present in the stomach. The rise in acidity is very slow. The secretion of the gastric juice, which is scanty after starvation, increases considerably 24 hours after food is introduced into the stomach. When the food has been in the stomach for a longer time (observations relate to only 72 hours after introduction of food) the gastric juice becomes more acid.

In all the specimens of *Cottus scorpius* and *Zoarces viviparus* examined for pH determinations, it is found that pH values are generally lower in the

anterior region of the stomach than in the posterior region. The average pH values in the anterior region of the stomach of *C. scorpius* as observed after starvation, and for 24, 48 and 72 hours after introduction of food, were 7·36, 5·72, 5·04 and 4·5 respectively; the pH values in the posterior region under the same conditions were 8·28, 6·84, 5·77 and 4·74 respectively. The average pH values in the anterior region of the stomach of *Z. viviparus* under the same conditions were 6·9, 5·8, 5·12 and 4·85 respectively. The pH values in the posterior region were 7·5, 7·0, 5·85 and 5·15 respectively. These differences in pH between the anterior and posterior regions of the stomach indicate that the former is more acidic than the latter.

It seems likely, therefore, that acid is secreted only in the anterior region, the posterior being acidic because it receives it from the anterior region. The two parts are in open communication in the short stomachs of the two species. Secretion of acid in the anterior region is quite in accordance with the histological observations that the gastric glands are found in the anterior region (= corpus) and are absent in the posterior region (= pylorus). As the other histological features of the two regions of the stomach in these two species are similar, it seems likely that the acid in the corpus is secreted by the gastric glands.

In the starved specimens of *C. scorpius* and *Z. viviparus* the anterior and posterior regions of the stomach also showed differences in pH, suggesting that acid was secreted despite absence of food from the stomach.

Various workers have shown that pepsin in fishes is produced in the corpus and not in the pyloric region (Yung, 1898; Sullivan, 1905; Herwerden, 1908; Beauvalet, 1933). This would mean, therefore, that both acid and pepsin are secreted by the gastric glands in the corpus. The gastric glands in fishes are not differentiated into oxyntic and peptic cells (Edinger, 1877). This has also been confirmed by later workers. My work on these two species has led to, also, similar results. If all the cells of the gastric glands are identical, then they must have the dual function of secreting both acid and pepsin.

On the other hand if the cells are physiologically different, then various chemical stimuli, such as histamine, acetylcholine and pilocarpine should bring about the qualitative change in the gastric juice which, in turn, would depend upon the unequal quantitative activity of two kinds of cells of the gastric glands. Dobreff (1927) found that there is no psychic phase in the gastric secretion in *Scyllium canicula*. He also found that intramuscular injection of pilocarpine, acetylcholine, atropine and histamine had no effect

on the secretion of the gastric juice and thus inferred that the control of gastric secretion must be humoral. Babkin, Chaisson and Friedmann (1935) did not find any evidence of nervous control of the secretion in *Raja diaphanus* and *R. erinacea*. Bayliss (1935) injected *Pleuronectes platessa* with pilocarpine and considered this was followed by increased secretion of pepsin. Although there are conflicting opinions on the factors controlling gastric secretion and more data are required, it may provisionally be suggested that the evidence now available supports the view that both pepsin and acid are secreted by the same cells.

Although in *Cottus scorpius* some of the food particles had passed from the stomach into the intestine between 48 and 72 hours after introduction of food, the lowest pH observed was 3·42 on a piece of soft flesh in the stomach for 72 hours. In *Z. viviparus* the lowest pH observed was 5·05 on the wall of the corpus, when the food had been in the stomach for 72 hours. From the pH values observed it seems, therefore, that there must not be much free HCl during gastric digestion.

The free HCl in the gastric juice is generally determined by titrating gastric juice with 0·1 N. NaOH using Topfer's indicator which changes the colour from red to yellow between pH 2·9 and 3·6. Most of the observations on pH determinations in the stomach of teleosts, including the present two species, would suggest that there is little free HCl.

Under the same conditions of artificial feeding in *C. scorpius* and *Z. viviparus* the rise of acidity in the former is more than in the latter.

The pH values on the food substances lying in the stomach are generally lower than the values on the underlying stomach wall.

The pH values in the pyloric cæca of *Cottus scorpius* are nearly the same as those in the duodenum. The duodenum has an alkaline reaction.

Acknowledgement

I acknowledge my indebtedness to Prof. A. D. Hobson, for his advice, freely given, on all occasions and for his guidance throughout my work. My thanks are due to Dr. H. J. Vonk, Director, Laboratory of Comparative Physiology, University of Utrecht (Holland), for extending the facilities of the laboratory.

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HARE-LIP AND CLEFT PALATE IN DOUBLE-MONSTERS

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Received March 12, 1953

A large volume of literature is available on the biology of identical twins from the genetic standpoint. The diagnosis of monozygotic twins though based on a number of anatomical characters establishes only the great probability of the twins being identical as a fairly reliable inference. But in cases of conjoint twins or double-monsters we have unimpeachable evidence of the twins being split portions of the same embryonic disc and hence absolutely positive factual evidence of identity in genetic make-up. The material available for such study, *i.e.*, double-monsters, is very scarce, and hence the following observations are considered worthy of publication.

The occurrence of hare-lip and cleft palate in both components of a double-monster was noticed in a specimen dissected by one of us in 1928 (Specimen 1, Fig. 1). Since then we have been on the lookout for a similar condition to gather additional evidence. Observations have been made on three cases of double-monsters in which both components show the abnormality of hare-lip and/or cleft palate. In all of them the defect is symmetrical so that one component is a mirror image of the other.

Report of Cases

Two of the specimens are human fœtuses and the third is a double-headed kid. Diagrams 1, 2 and 3 show the appearance of the specimens.

Specimen 1.—The specimen is a human double-monster, female, thoracopagus tetrabrachius tetrapes. The union is extensive on one flank from the upper part of the thoracic region to umbilical region. Both faces show unilateral hare-lip and cleft palate, the right component on its left side and the left component on its right side, so that each is a mirror image of the other.

Specimen 2.—This is a human monster with single trunk and a fused double head showing two faces, diprosopus distomus Triophthalmus, diotus. Each face shows a bilateral hare-lip with cleft palate.

Specimen 3.—This is a double-headed male kid with cleft palate in both components. The palatal cleft in each is median with defective nasal septum which is a sickle-shaped ridge at the roof of the nasal cavity.

It is not necessary for the purpose of this paper to give a detailed description of the abnormality in the specimens as the significant fact is the symmetrical occurrence of the anomaly in both components and especially the mirror imaging of the unilateral anomaly in the first specimen.

DISCUSSION

Among the various etiological factors for the conditions, hare-lip and cleft palate, the following are usually cited.

1. Local factors like amniotic bands preventing the normal union of the processes constituting the face or non-union due to circulatory failure (Keith, 1940) or pressure caused by the lower jaw and tongue forcing out the processes forming the upper lip or palate in instances where the hyperflexed chin abutted against the chest or knee (Cryer, cited by Mead, 1946).

2. Injury to a part of the embryonic mozaic due to environmental factors at a critical stage in early development leading to defect or malformation of the part of the body derived therefrom (Stockhard, Ballantyne, cited by Willis, 1950; and Murphy, 1947).

3. Hereditary factor transmitted as a mendelian inheritance (Gruneberg, cited by Browne, 1950; Gates, 1946, and many others).

In individual cases where the presence of amniotic bands or local causes leading to pressure on or necrosis of embryonic parts have been definitely made out, it cannot be denied that such factors do have an etiological bearing. But in the instances presented here, showing symmetrical deformity of the two components, purely local factors could reasonably be eliminated.

There is ample and accumulating evidence concerning the correlation between defective development and anoxia, chemical poison, or bacterial or viral toxin present in the environment at critical stages of early embryonic development. If such factors are considered as being operative causes in the instances of double-monsters presented here, one would naturally expect a bilateral defect in each of the components of the double-monster because the facial processes that go to form the upper lip of the embryo appear synchronously and progress to their fusion simultaneously on the right and left side of the embryo. Such a hypothesis may explain the condition in

Specimens 2 and 3 but not the condition in Specimen 1. There is no evidence yet to assume that the stages of embryological development on the right and left halves of the upper lip are asynchronous and that one lateral half of the upper lip is constituted in advance, while the other half lags behind in development. The absence of a bilateral defect in both components in Specimen 1 would eliminate the possibility of toxic influence at a critical stage during development as a causative factor.

Further, the fact that the anomaly is subservient to the law of symmetry of the total embryonic primordium composed of both components, *i.e.*, the fact that one component is a mirror image of the other in unilateral asymmetry would lead us to infer that we have to deal here with an inherent tendency of the embryo to develop in a malformed manner. This tendency conduces to the production of an anomaly of a particular kind, but not necessarily focussed to any one particular antimeric side of the embryo. In its ultimate expression this tendency is seen to be conditioned by the compelling law of symmetry as applicable to the total embryonic area, as if an axial line for symmetry were passing through the conjoint portion of the double-monster. Such a tendency could only be a genetic factor. In such a case it could vary in its penetrance and expression from parent to offspring and even on the two sides of the individual. Being carried by the genes in the chromosomes its transmission from one generation to another would be in the manner of mendelian inheritance.

The symmetrical occurrence of unilateral hare-lip and cleft palate in a double-monster is an additional evidence supporting the genetic factor in the etiology of these conditions.

Acknowledgment

We are thankful to Col. V. Srinivasan, erstwhile Principal, Medical College, Nagpur, for photograph of Specimen 2.

Summary

Three cases of hare-lip and/or cleft palate occurring in both components in double-monsters in a symmetrical manner are recorded. One of the specimens shows unilateral hare-lip and cleft palate occurring on the near side of the two components, each mirror imaging the other in its individual asymmetry. This adduces additional evidence to the genetic factor in the etiology of these conditions.

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FIG. 1. Human double monster showing unilateral hare-lip and cleft palate occurring symmetrically.

FIG. 2. Human double-headed monster showing bilateral hare-lip und cleft palate occurring symmetrically.

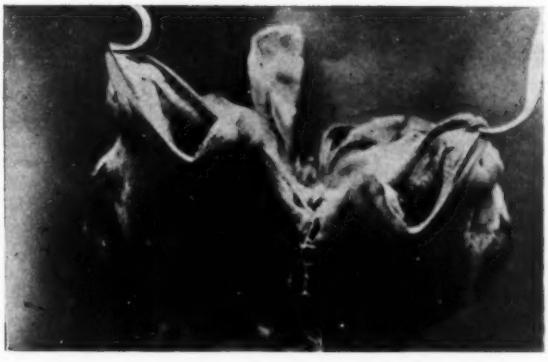

FIG. 3. Double-headed kid showing cleft in palate occurring symmetrically.

BACTERIUM OXALATICUM, A NEW OXALATE-DECOMPOSING BACTERIUM ISOLATED FROM THE INTESTINE OF EARTHWORMS

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Received May 19, 1953

(Communicated by Dr. P. Maheshwari, F.A.SC.)

Introduction

Of the few bacterial species known to decompose oxalate, practically all belong to the *Pseudomonadaceæ* family. This may be readily seen from the fact that the oxalate-decomposer described by Bhat and Barker (1948) was a *Vibrio* species, and the *Bacillus extorquens*, a large, bent, non-sporulating rod isolated by Bassalik (1913) from earthworm excreta, though named *Bacillus*, should, taxonomically speaking, be placed in the genus *Vibrio*. Again, *Pseudomonas rimæfaciens*, described in the *Bergey's Manual* (Breed *et al.*, 1948) as capable of utilizing oxalate, belongs to this family. In our own laboratory, Pavri and Bhat (1953) had obtained, in oxalate enrichments of leaf galls of some plants, another *Pseudomonas—Ps. astragali*. Recently, Khambata (1952), Khambata and Bhat (1953) have described the isolation of *Ps. oxalaticus*, a new species of an oxalate-decomposing *Pseudomonas*.

The purpose of this paper is to describe the isolation and characteristics of a new oxalate-decomposing micro-organism—*Bacterium oxalaticum* and to show that the bacterial degradation of oxalate is no longer a sole monopoly of the *Pseudomonadaceæ* family.

Experimental

Isolation of Bacterium oxalaticum.—Freshly collected earthworms, after being killed by chloroform, were surface sterilized by treatment in a mercuric chloride disinfectant. Traces of the disinfectant were washed off in sterile water. Then the first fourteen segments were aseptically snipped off and the remaining portion constituting the intestine was mashed up in sterile physiological saline so as to obtain a uniform suspension of the intestinal contents. A detailed description of the procedure can be obtained elsewhere (Khambata, 1952; Khambata and Bhat, 1953). One ml. of the suspension was then inoculated into Erlenmeyer flasks containing Bhat and Barker's oxalate medium (1948) slightly modified by the addition of 0·1% yeast

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autolysate. The flasks were incubated aerobically at room temperature (28–30° C.). After enriching the culture three or four times, it was finally streaked on oxalate agar to which was added 2 ml. of sterile calcium chloride solution per 100 ml. of the medium. After 4 days incubation at room temperature colonies with haloes around them appeared due to the dissolution of calcium oxalate crystals. They were then picked up and purified as per the method of Bhat and Barker. The pure cultures were maintained on oxalate agar and on plain nutrient agar.

Researches conducted during the course of three years on the intestinal microflora of earthworms have resulted in the study of (besides the pseudomonads already described) two strains of a bacterium capable of utilizing oxalate as well as formate. The two strains are almost identical morphologically and culturally but differ in minor respects only in their ability or otherwise to utilize certain compounds as sole sources of carbon and energy.

Morphology and Staining.—Short, gram-negative, plump, coccoidal rods ($1 \cdot 0 \mu \times 1 \cdot 1 - 1 \cdot 3 \mu$) in pairs and in clumps (Plate III, Fig. 1). Non-sporulating, non-motile, but capsulated.

Cultural Characteristics.—On nutrient agar, after 2 days, 0·5 mm. round, smooth, opaque, slightly fluorescent, convex, entire-edged colonies are seen. After 5 days the growth becomes slightly mucoid. In nutrient broth, the organism forms a thin pellicle, turbidity and a membranous sediment. On Bhat and Barker's oxalate agar, after 5 days, the colonies are about 0·75 mm. round, smooth, opaque, white, slightly fluorescent, convex, entire-edged, and with distinct haloes around them (Plate III, Fig. 2). Due to the utilization of anions the pH of the medium becomes alkaline and the phenol red indicator in the oxalate medium imparts a red colour to it. In liquid oxalate medium, a membranous growth develops at the bottom of the tube, and sometimes also along the sides. Once again, the medium turns red with the utilization of oxalate.

The bacterium reduces nitrate to ammonia after 7 days, although tests made on the 1st, 3rd and the 7th day for reduction to the nitrite stage have given negative results in our hands. It does not form indole. Hydrogen sulphide is produced in very small quantities after 12 days by one of the two strains (strain Ox_7). Strain Ox_5 reduces litmus milk within 12 days and after 30 days shows peptonization with acidity which is observable only on shaking the tube. Strain Ox_7, however, produces strong alkalinity in litmus milk within 2 days. Gelatin is not liquefied. M.R. and V.P. tests are negative. Catalase is formed. No acid or gas is produced in glucose, lactose, saccharose, maltose, starch, dextrin, glycerol or mannitol.

Substrates Utilized for Growth.—Using ammonium sulphate as the nitrogen source in a mineral solution, various carbon compounds were tested for their suitability or otherwise as growth substrates for *Bact. oxalaticum*. The two strains of this organism showed slight differences in their capacity to utilize certain carbon compounds, as can be observed in Table I.

TABLE I

Strain No.	Utilization of										
	Oxalate	Formate	Acetate	Succinate	Lactate	Tartrate	Citrate	Ethyl Alcohol	Glucose	Dextrin	Starch
Ox_5	+	++	+*	−	++	+	−	+	+++	+++	+++
Ox_7	+	++	−	−	+++	−	−	−	+	+	−

+++ Good growth
++ Fairly good growth
+ Poor growth
+* Poor growth after 5 days
− No growth

To test the ability of the bacterium to utilize various nitrogen sources, the nitrogen levels were maintained on the basis of 25 mg. per 100 ml. of the mineral solution containing 1% sodium lactate as the sole carbon source. Both strains were unable to utilize atmospheric nitrogen and ammonium nitrate. Urea utilization was variable. Ammonium sulphate, sodium ammonium phosphate, diammonium hydrogen phosphate and potassium nitrate were poorly utilized by them, while sodium aspartate and sodium glutamate yielded fairly good growth. Peptone was fairly well utilized by strain Ox_5 but for strain Ox_7 it proved to be a poor nitrogen source.

Taxonomy.—This gram-negative, non-sporulating, capsulated coccobacterium cannot be placed in any of the homogeneous species collection placed in a family. Obviously they belong to the heterogeneous collection of species whose relationships to each other and to other groups we are not aware, and consequently as Breed *et al.* (1948) have proposed, we are placing it in the single genus *Bacterium* of the family *Bacteriaceæ*. Apart from the fact that it can be characterized by its ability to utilize oxalate as well as formate as sole sources of carbon, it appears to represent a distinct species

from all the 56 species described in the *Bergey's Manual* as judged from one or more of the significant features which we exploit in the differentiation of species placed in this genus, and hence we propose the name *Bacterium oxalaticum* nov. sp. for it. The two strains differ slightly in some nutritional aspects but this we regard as a difference in the strains rather than in the species.

SUMMARY

The isolation, cultural characteristics and the nutrition of an oxalate-decomposing micro-organism, *Bacterium oxalaticum* nov. sp., obtained from the intestine of Indian earthworms, are described and its taxonomical position discussed. Besides utilizing oxalate as a sole source of carbon the bacterium can utilize formate even better. Attention has been drawn to the fact that oxalate-decomposing bacteria occur in a family other than the *Pseudomonadaceæ*—a family which had hitherto yielded practically all the known oxalate-decomposing bacteria.

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EXPLANATION OF PLATE

FIG. 1. Photomicrograph of *Bact. oxalaticum* showing coccobacteria arranged in pairs; from a 24-hour old culture on nutrient agar. Gram stain, × 900.

FIG. 2. Five-day-old culture of *Bact. oxalaticum* on Bhat & Barker's oxalate agar. Decomposition of the oxalate is indicated by the clearance of calcium oxalate around the colonies (2½ times enlarged).

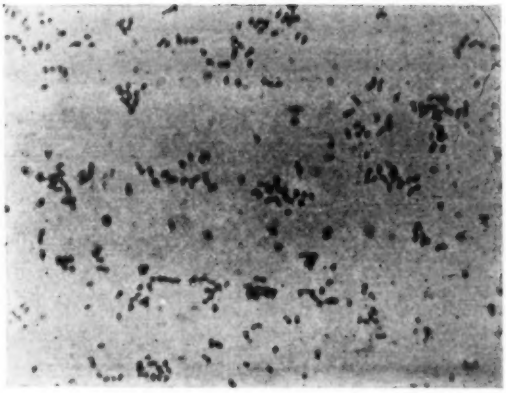

FIG. 1

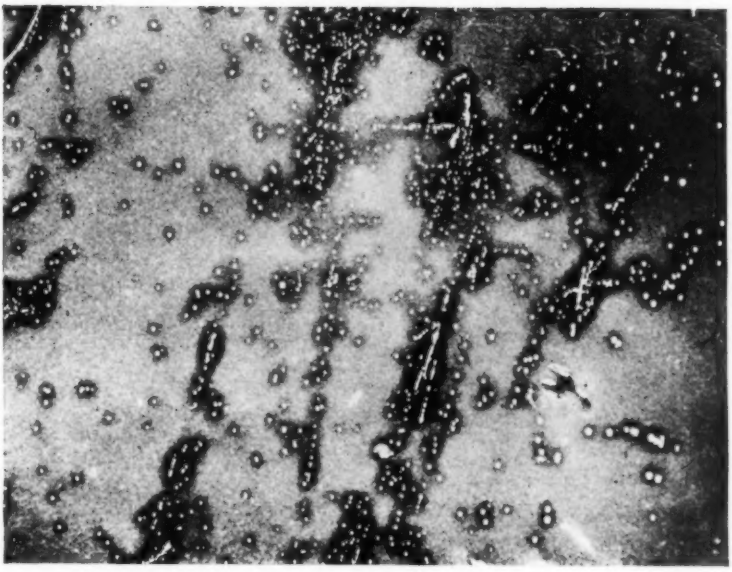

FIG. 2

MECHANISM OF FUSARIUM WILTS OF PLANTS

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Received July 3, 1953
(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

In a recent report Scheffer and Walker (1953) have presented experimental evidence and compared the toxin theory of wilt with the theory of vessel plugging by fungal growth with special reference to tomato wilt by *Fusarium oxysporum* f. *lycopersici*. Employing neat culture filtrates on cut shoots they have observed that regardless of age of the culture no vascular browning or plugging occurred in 24 hours but only leaf-blade injury while the diluted filtrates showed vascular browning and plugging in 3 days and that all plants died of soft rot by that time. It is necessary to point out that the neat culture filtrates may not produce wilt symptoms exactly comparable to the natural wilt in view of the various other toxic (or interfering) metabolic products, other than the specific toxin(s) and also the unmetabolised ingredients of the medium. In a recent note from this laboratory (Kalyanasundaram and Lakshminarayanan (1953), the use of dialysed culture filtrates of *F. vasinfectum* to produce toxic symptoms in cut shoots of cotton has been indicated since it would be thus possible to eliminate most of the other toxic metabolites, while the specific toxin(s) itself is intact. It may be pointed out that the technique of replacement culture which Scheffer and Walker (*loc. cit.*) have employed is not likely to eliminate the effect of other toxic metabolites and the unmetabolised ingredients of the medium. It is probable that there are more than two factors in the culture filtrates of *F. oxysporum* f. *lycopersici*, one responsible for vascular browning and the other connected with leaf-blade injury, etc. It is of interest to mention in this connection that two non-dialysable factors have been observed to be present in culture filtrates of *F. vasinfectum*—a thermolabile toxic factor and a thermostatic factor promoting root production and that they are mutually antagonistic (Kalyanasundaram and Lakshminarayanan, *loc. cit.*). Wellman (1943) has shown that while filtrates from freshly growing cultures of *Fusarium bulbigenum* were severely toxic to tomato leaves and tip buds, it was only aged culture filtrates that produced severe leaf-blade injury and stem collapse and that further ageing considerably reduced the toxicity. It is likely that similar factors and antagonisms exist in the case of *F. oxysporum* f. *lycopersici* also. In view of the exo-cellular nature of the toxin(s)

it is not likely to be retained in the replacement culture technique and naturally it would take greater time for the replaced culture media to attain the toxic status with the washed mycelia and even then the interference of the unmetabolised ingredients of the media would still be there. Since the authors themselves claim that the toxin is quite stable after 15 hours' dialysis, it is suggested that the dialysed culture filtrates would yield more comparable results than those with the other technique.

It would be relevant to observe in this connection that while Gäumann and his school of workers (Gäumann, 1951; Gäumann and Jaag, 1947, 1950; Gäumann *et al.*, 1950, 1952) have been advancing the toxin theory of wilting, the other school of Walker (Scheffer, 1952; Walker and Foster, 1946; Scheffer and Walker, 1953) has been largely supporting the vessel plugging theory. Scheffer and Walker (*loc. cit.*) attribute vessel plugging as the immediate cause of wilt since no toxin could be established in the wilting plant. Nevertheless, Gottlieb (1944) has reported conclusive evidence establishing the presence of toxin in tracheal fluids of wilt infected tomato plants by *Fusarium bulbigenum* var. *lycopersici* and indicated that while a dense mycelium was present in the roots and lower parts of the stem, no fungal growth could be detected in the upper parts; in no case was there enough plugging to account for wilting. The recent interesting experiments of Brian (1952) on the uptake of antibiotics like griseofulvin from the rhizosphere region and their translocation to the guttation fluid and the relative stability of phytotoxins in the soil indicated by Jefferys' work (Jefferys, 1952) afford overwhelming evidence in support of the toxin theory. The possibility of one of the toxins—fusaric acid, being produced in sufficient concentrations in the soil and translocated to the other parts has been envisaged in Gäumann's recent report (Gäumann *et al.*, 1952), wherein they have mentioned the possibility of a third toxin other than fusaric acid and lycomarasmin, probably responsible for the browning in tomato leaves. In this laboratory Satyanarayana (1953) has shown that the heat inactivated dialysed culture filtrates of *F. vasinfectum* (containing the growth-promoting factor) can inactivate the toxin(s) *in vivo* when added to the soil along with the fungal inoculum and maintained at a constant level. This finding lends further support to the theory of systemic translocation of the toxin(s).

It should, however, be emphasised that what happens in the cases of natural wilting can be satisfactorily explained only by a combination of both the theories. The toxin(s) is probably only one of the metabolic products formed by the action of the exo- and endo-cellular enzymes of the pathogen on the susceptible host. The toxic symptoms or disease syndrome

such as leaf-blade injury, browning, vessel plugging, etc., represent only some of the aspects of the natural wilt which is more complicated than the condition of *in vitro* toxemia. As such the susceptibility or resistance of the host depends both on the chemical set-up of the host and the conditions under which the enzymes and substrates react. The virulence of the parasite is probably governed by its enzymic set-up. The other aspect of wilting, *viz*., the endo-cellular enzyme systems of the parasite, has not received enough attention from the toxin school of workers but has been partly emphasized by the vessel plugging theory. The active fungal cell has to be present at the site of reaction in order that the endo-cellular enzymes might come into play. The respiratory and other changes brought about in the host during wilting are probably connected with the endo-cellular systems and the vessel plugging theory suggests evidence in favour of these. It would thus be evident that a judicious combination of both the theories only could explain satisfactorily many of the anomalies so far observed as the susceptibility of cut shoots of resistant varieties of hosts to the action of toxins like lycomarasmin and the production of increased quantities of toxin by relatively avirulent strains of Fusaria.

A detailed study of enzyme systems of Fusaria is under way and will be published in due course.

Acknowledgements

I am greatly indebted to Professor T. S. Sadasivan for guidance and to my colleagues, Dr. C. V. Subramanian and Mr. R. Kalyanasundaram, for discussions.

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STUDIES IN THE INDIAN AQUATIC FUNGI*

I. Some Water Moulds of Lucknow

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The record of the Aquatic Phycomycetes of India is rather meagre. A scrutiny of the literature shows that only a few forms of the Chytridiales, one of the Blastocladiales and one of the Lagenidiales have so far been reported from this country. Saprolegniales and Peronosporales have, however, received a greater attention (Chaudhuri *et al.*, 1951). Aquatic fungi, therefore, appeared to be a profitable field of study and the authors attempted to make an exhaustive collection of the various aquatic fungi by employing the usual methods of collection as given by Sparrow and other workers. A large number of forms belonging to the different orders of this group thus obtained are now under investigation. The observations herein described pertain to three well-known groups of Aquatic Phycomycetes, namely, Blastocladiales, Monoblepharidales and Lagenidiales. All the species described except Myzocytium have apparently never before been reported as occurring in India. It is necessary, however, to note that this paper is taxonomic and that no attempt has been made here to give a detailed account of the development and life-history of each fungus described. The identifications are provisional.

Blastocladiales

Among the large number of forms collected the majority belongs to the genus Blastocladia. Four of these conform to the species already known and they are described here. The rest await specific identification.

(1) *Blastocladia sparrowii* Indoh.

Basal cell cylindrical, 200 μ long by 80 μ in diameter, wall smooth, expanded distally giving rise to cylindrical subdichotomously arranged lobes, setæ absent. Hold fasts branched; the whole plant 450–500 μ long, Sporangia borne over the surface of the lobes, of two types, bigger empty ones and smaller ones filled with contents, the smaller ones cylindrical, tips narrow, curved 60–90 μ long by 20–25 μ in diameter; bigger ones cylindrical

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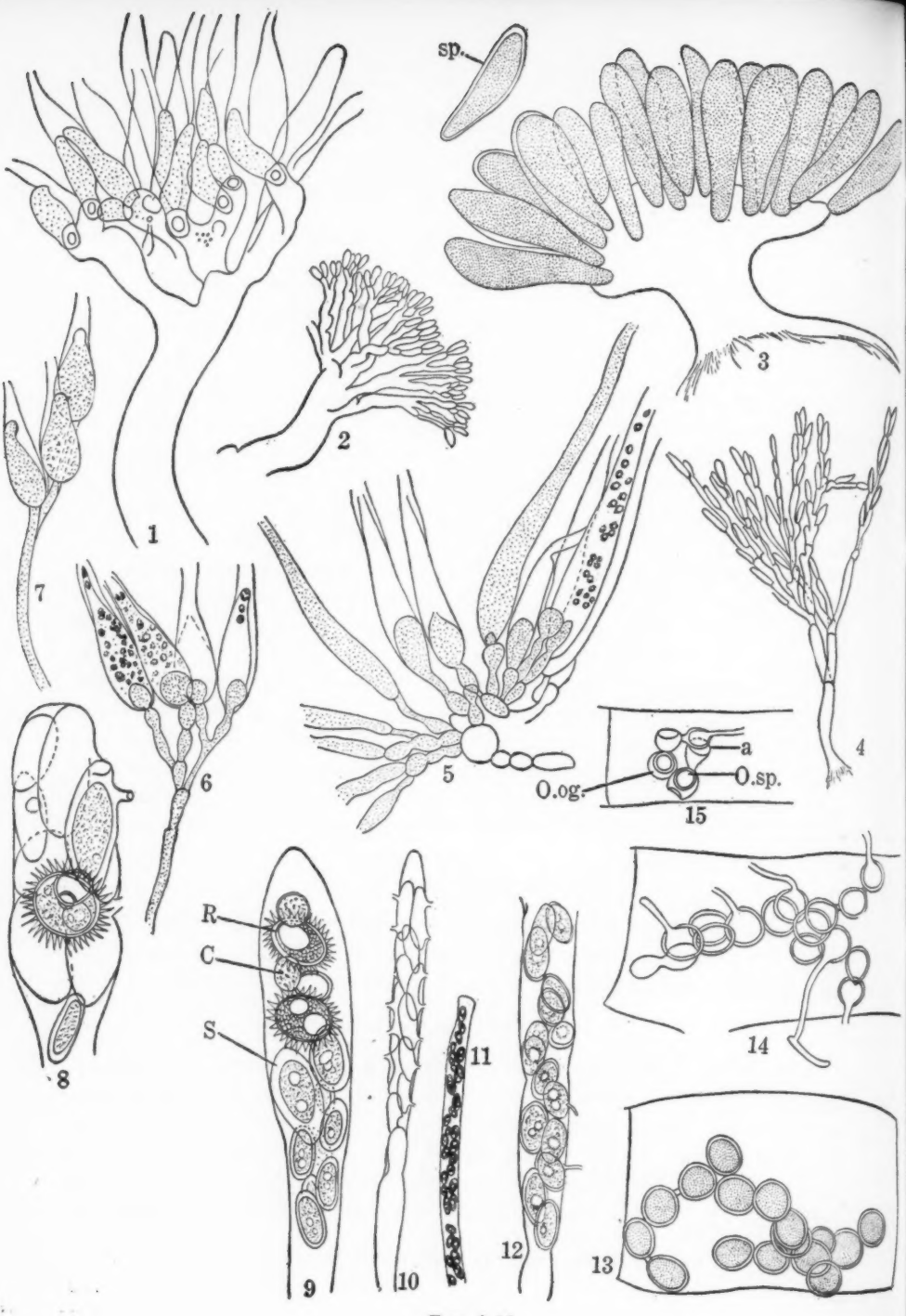

FIGS. 1–15

FIGS. 1–4 : Blastocladia

Fig. 1. *B. Sparrowii* Indoh., × 192.

Fig. 2. *B. ramosa* Thaxter, × 72.

Fig. 3. *B. globosa* Kanouse, × 192.

Fig. 4. *B. rostrata* Minden, × 72.

FIGS. 5–7 : Gonapodya

Fig. 5. *G. prolifera* (Cornu) Fischer, × 260.

Fig. 6. *G. polymorpha* Thaxter, × 260.

Fig. 7. Same as 6 showing hypha without constrictions, × 260.

FIGS. 8–12 : *Olpidiopsis achlyæ* McLarty

Fig. 8. Zoosporangia and resting spores in a terminal filament of Achlya causing swelling of the host, × 260.

Fig. 9. Same showing two resting spores R with companion cells C and Zoosporangia S, × 260.

Fig. 10. Same showing a number of discharged sporangia, × 80.

Fig. 11. Part of a filament with small oval Zoosporangia, × 80.

Fig. 12. Magnified view of Fig. 11, × 260.

FIGS. 13–15 : *Myzocytium proliferum* Schenk.

Fig. 13. A chain of bead-like sporangia in a cell of *Spirogyra* sp., × 260.

Fig. 14. Another cell of *Spirogyra* sp. showing discharged sporangia with discharge tubes × 260.

Fig. 15. Cell of *Spirogyra* sp. showing antheridia (*a*), oogonia (*oog*) and oospore (*os p*), × 260.

the narrow tips having prominent apical papillæ 110–140 μ long by 23–30 μ broad. Resting spores not observed.

On submerged fruits.

(2) *Blastocladia ramosa* Thaxter

Habit tree-like. Basal cell cylindrical 250–300 μ long by 90 μ in diameter divided distally subdichotomously into several secondary axes which branch and rebranch profusely, hold fasts delicate, sparingly branched, wall thin, smooth, setæ absent, whole thallus with an open ramose habit upto 570 μ long. Sporangia terminal and subterminal on short branches appearing like ear of wheat, long cylindrical, empty sporangia persisting, 32 45 μ long by 10–16 μ broad, papilla apical with an apical pore, zoospores spherical. Resting spores not observed.

On submerged fruits.

(3) *Blastocladia globosa* Kanouse

Basal cell globose, proximal cylindrical part very short 70 μ long by 70 μ broad, distally entire or with large irregular expanded lobes, wall smooth, contents brownish, hold fasts stout, branched, setæ absent, whole plant 260 μ

long. Sporangia broadly clavate 90–190 μ long by 32–40 μ broad, numerous, Resting spores not observed.

On submerged fruits.

(4) *Blastocladia rostrata* Minden

Habit slender, long, tree-like. Basal cell narrowly cylindrical 200 μ long by 32 μ in diameter, distally branching dichotomously, branches cylindrical throughout, holdfasts quite thick, branched, setæ absent, whole plant 850 μ long. Sporangia alternately arranged along the branches, on short protuberances, the younger ones being at the top. Empty sporangia persistent long and cylindrical with an opening at the tip. 60–75 μ long by 12–20 μ broad. Young sporangia club-shaped upto 40 μ long. Resting spores not observed.

On submerged fruits.

MONOBLEPHARIDALES

A large number of forms all of which belong to the genus Gonapodya has been collected. The variations and intergradations in the species of Gonapodya are known to be so extensive that Sparrow (1943) and other workers consider specific identification almost impossible. Therefore no attempt has been made to divide these forms into more than the two species already known, namely, *G. prolifera* and *G. polymorpha.*

(1) *G. prolifera* (Cornu) Fischer

The mycelium is profusely branched and the branches diverge from a common base. The hyphæ are divided by pseudo-septa or constrictions into clavate segments. The sporangia vary greatly in size and shape. They are very long and pod-shaped with an inflated bottom and a very elongate distal portion and it is the distinguishing feature of *G. prolifera* from *G. polymorpha.* They are either sessile, or separated from the terminal cell by a constriction or pseudoseptum, size 70–210 μ long by 15–20 μ in diameter. The plants are white to dark brown in colour. Zoospores with numerous small granules at the apex. Sexual reproduction not found.

On submerged fruits.

(2) *G. polymorpha* Thaxter

The thallus is filamentous and sparingly branched. The hyphæ are usually constricted at intervals and in these places pseudo septæ are formed. The segmented portion is sometimes borne on slender hyphæ in which segmentation is indistinct or absent. In a few cases hyphæ without any

constriction were found. Even at the end where sporangia were borne the constrictions seem to be absent. The protoplasmic contents show foamy or reticulate nature. At the ends the constrictions become rounded and ovate sporangia are borne on these. They vary from 50–100 μ in length by 18–30 μ in diameter. The sporangia may be terminal and solitary or forming fascicles. They do not have elongated distal portion like *G. prolifera* but taper abruptly to a blunt tip. They are once to many times proliferous. Zoospores many in a single sporangium, similar to those of *G. prolifera*. Sexual stage not found.

On submerged fruits.

LAGENIDIALES

Two members of this group, one belonging to the family Olpidiopsidaceæ and the other belonging to Lagenidiaceæ are described here.

Olpidiopsidaceæ

(1) *O. Achlyæ* McLarty

Thallus is endobiotic and characterised by the absence of a vegetative system. In the young stages the thallus seems to be naked but later on it has a definite wall. The whole thallus forms a single reproductive structure and the contents have refractive globules. Zoosporangia are one to many found either in a terminal part of the host filament or intercalary. The parasite may or may not cause swelling of the host filament. Zoosporangia are smooth, spherical, oval or elongate and varies greatly in size. 30–120 μ long by 12–36 μ in diameter. Usually one and sometimes two exit tubes are produced which may extend beyond the surface of the host filament. Zoospores could not be observed. Resting spores are spherical or oval and are formed sexually or asexually. These also contain refringent globules and have two walls. The endospore is smooth but the exospore has narrow or broad spines or has a wavy margin. Some were found to be smooth also. 25–45 μ by 30–50 μ. Male cells look like zoosporangia, 20–30 μ by 15–25 μ, and one or two are attached to one female cell. They are either oval or round and have smooth walls. On germination the resting spores act like zoosporangia. They produce exit tubes through which the zoospores escape. Discharge tube 7–12 μ by 3–7 μ.

Parasitic in Achlya sp.

Lagenidiaceæ

(2) *Myzocytium proliferum* Schenk.

Thallus elongate and constricted consisting of 1–15 μ segments in a linear series. Sporangia hyaline, smooth, spherical, 18–22 μ long by 16–22 μ

wide. Each sporangium has two walls and forms a single cylindrical discharge tube which may extend considerably beyond the wall of the host. Gametangia occurring among the zoosporangia, oogonia ellipsoidal, antheridia fusiform 18–35 μ by 10–20 μ, oospores spherical 20–32 μ long by 10–20 μ broad with a double wall and a large refractive globule, lying loosely in the female gametangium. Germination not observed.

Parasitic in Spirogyra.

This has already been reported from the Punjab by Chaudhuri (1931), but it is a new record for U.P.

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STUDIES ON THE MICRO-ORGANISMS ASSOCIATED WITH THE LEAVES OF *PONGAMIA GLABRA* VENT.

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OUR knowledge of bacterial association with plants dates from the work of Hellriegel and Wilfarth (1888), who revealed the beneficial influence of bacteria on leguminous plants on whose roots they grow, enriching the soil in which these plants are grown. However, the most rapid advancement regarding the bacterial diseases of the plants or the so-called 'malevolent associations of the bacteria with the plants' has been made only during the past 35 years.

Establishment of the fact that certain plant diseases are definitely caused by bacteria alone, led to descriptions of many plant diseases, the causes of which had been unknown previously. A glance at the 6th edition of *Bergey's Manual* (1948), will reveal approximately some 170 species of bacteria now recognized to be pathogenic to plants. Detailed descriptions of different specific bacterial diseases and pathogens can be obtained from the works of Smith (1905, 1911, 1914 and 1920), Elliot (1930), Stapp (1935), Levine (1931, 1936 and 1940), Grieve (1943), Riker (1940 and 1942), Riker and Berge (1935), Riker, Gutsche and Spoerl (1946), Hillderbrandt (1950) and several others.

However, the information available, regarding the association of micro-organisms with the leaves of plants, is not very adequate in spite of the notable efforts made by the earlier investigators; and hence, it was felt worthwhile to study this problem, especially from the point of view of some of the tropical plants which abound in the so-called gall-formation on leaves.

This paper deals with the studies made in connection with the micro-organisms associated with the leaf-galls encountered on *Pongamia glabra* Vent., a plant, distributed throughout the length and width of India.

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MATERIAL AND METHODS

The material utilized throughout this study, was collected from Bombay and Salsette islands during the years 1948–50. Care was taken to employ fresh leaves showing gall-formations at different seasons of the year to ascertain if there was any seasonal variation in microflora. For experimental purposes, mature, well-sized galls were generally made use of, but for informative purposes, early and late formations were also examined occasionally. The carefully cut galls were surface-sterilized in a mercuric chloride solution (Harrison and Barlow, 1907), then rinsed in several changes of sterilized water and carefully crushed in a sterile petri-dish with the aid of a sterile scalpel and then suspended in sterile water; this was then used as the inoculum, after making it up to a definite standard opacity. For the cultural work, the following routine media were employed:

Meat infusion agar, pH 7·2–7·4;
Sabouraud's agar, pH 5–6;
Ashby's mannitol agar, pH 7;
Leaf extract agar, pH 7;
Carrot extract agar, pH 7–7·2;
Yeast-autolysate congo red agar, pH 7; and
MacConkey's agar, pH 7·6.

However, later on, after some careful investigation, it was found that so far as the material under examination was concerned, the use of all these media was not called for and hence, in subsequent work, we stopped using Ashby's agar, carrot extract agar, leaf extract agar and MacConkey's agar.

Enrichment media.—Besides using the abovementioned media, we also employed the enrichment culture methods. It was Beijerinck (1921–40) who, by introducing the principle of enrichment cultures, opened the way for a rational approach to microbial ecology. To explain them in his own words, " enrichment cultures in reality, are nothing but the broadest application of the elective cultural method and the method is fruitful and truly scientific; it is no exaggeration to claim that the rapid and surprising advances in general microbiology are due to this methodology ".

In our investigations, we had to take into consideration the metabolic transformation taking place in a carbohydrate molecule, for after all we were concerned with the study of micro-organisms associated with the leaves of plants, which obviously are factories, wherein carbohydrate chemistry is being worked out in a unique manner. Consequently, the various intermediaries in the build-up or the break-down of the starch molecule had to

be carefully examined for their relative values as substrate-materials for obtaining enrichment cultures. After careful consideration, we chose oxalic acid $(COOH)_2$, formic acid (HCOOH), lactic acid ($CH_3CHOH.COOH$), malic acid ($HOOC.CH_2CHOH\ COOH$), fumaric acid ($HOOC.CH=CHCOOH$), succinic acid ($HOOC.CH_2CH_2\ COOH$), citric acid ($HOOC.CH_2COH.COOH.CH_2COOH$), glucose ($C_6H_{12}O_6$) and starch $(C_6H_{10}O_5)_n$ as substrates for these culture methods.

Generally speaking, a basal solution of the following composition, made with extra-pure chemicals, was employed for the preparation of several media composed of substrates named above.

Basal Solution

Distilled water	2000 ml.
Ammonium sulphate	1·00 gm.
Magnesium sulphate	0·20 gm.
Dipotassium hydrogen phosphate ..	1·00 gm.
Ferrous sulphate	0·02 gm.
Saturated solution of calcium sulphate ..	10·0 ml.
Yeast-autolysate	10·0 ml.
Phenol red (B.D.H.)	10·0 ml.

All these media were employed in liquid form and attempts were made to isolate both aerobic and anaerobic bacteria from the galls of plants.

Results

By application of the methods described above, altogether 33 isolates were obtained as a result of 6 analyses; these isolates could be, from preliminary observations, grouped into two types inasmuch as one group consisted of gram-negative short rods and the other gram-positive cocci. Numerous physiological and biochemical tests were carried out for identification of these 33 isolates; but as they are all routinely employed in all laboratories they need no special mention here. Tables I and II indicate several nutritional tests done for further confirmation of the identities of these organisms. Of these isolates, 15 were specifically the same, even though some slight differences, initially significant enough, were observed; these are labelled as 1 *a*, 1 *b* and 1 *c* in the tables. Of the remaining 18, some are labelled as No. 2 and others as Nos. 3, 4 and 5.

The final identification was achieved by reference to 6th edition of *Bergey's Manual* (1948). Accordingly, they were found to be (1) *Pseudomonas æruginosa*; (2) *Pseudomonas arvilla*; (3) *Pseudomonas astragali*; (4) *Pseudomonas hydrophila*; and (5) *Micrococcus citreus*.

TABLE I

Utilization of Carbon Sources

Isolates Nos. ( )	Bicarbonate	Oxalate	Formate	Acetate	Butyrate	Lactate	Malate	Succinate	Tartrate	Citrate	Glycerol	Ascorbate	Glucose	Fructose	Maltose	Dextrin	Starch
1 *a*	–	+ (Scanty)	++	*a +++	*a +++	++++	++	*a +++	–	++	++	–	++	+	+	+	+
1 *b*	–	+ (Scanty)	++	++	*b ++++	*b +++	++	*b +	–	++	++	–	++	+	+	+	+
1 *c*	–	+ (Scanty)	++	++	*c +++	*c +++	++	*c +++	–	++	++	–	+	+	+	+	+
2	–	+ (Scanty)	++	++	+++	++++	++	++	–	++	++	–	+	+	+	+	+
3	–	+	+	++	+	++	–	++	+	++	+	+	++	+	++	++	++
4	–	–	–	+	+	*e +++	++	+	++	*e ++++	++	–	++	++	++	++	++
5	–	+ (Scanty)	+ (Scanty)	+ (Scanty)	–	+	–	+	+ (Scanty)	–	+	–	+	+	+	+	+

Legend: ( ) These results are representative for all the 33 isolates

++++ Indicates excellent growth

+++ ,, good growth

++ ,, fairly good growth

\+ ,, poor growth

*a Pale yellow green

*b Chrysopase green

*c Tyrolite green

*d Greenish yellow

*e Bright golden yellow

TABLE II

*Utilization of Nitrogen Sources**

Isolate Nos. X	Ammonium sulphate	Sodium nitrrate	Sodium nitrite	Peptone	Tryptone	Meat extract	Urea	Casein hydrolysate	Sodium caseinate	Yeast autolysate
1*a*	+++	++	++	+++	++++	++++	+	++	+++	+++
1*b*	+++	+	+	++	++++	++++	+	+	++	++
1*c*	+++	++	++	+++	++++	++++	+	+	++	++
2	+++	−	++	+++	++++	++++	+	+	++	+++
3	+	−	−	++	++	++	−	++	+	++
4	+++	−	++	++++ **	++++ **	++++ **	−	+++	+++	−
5	−	−	−	+	+	+	−	−	−	−

Legend : X These results are representative for all the 33 isolates

** The limited growth of *M. citreus* is due to limited supply of Peptone, Tryptone and Meat extract which did not exceed 100 mg. %

++++ Indicates excellent growth

+++ „ good growth

++ „ fairly good growth

\+ „ poor growth

Table III indicates the isolates obtained from the enrichment culture media. Aerobic methods resulted in successful isolation of all the four species of *Pseudomonas* and it is pertinent to mention here that their isolation was achieved with much more ease by this method than by the application of the routine culture methods. It is extremely interesting to observe that the oxalate cultures were purely made of *Pseudomonas astragali* and no other species, whilst citrate and fumarate yielded pure cultures of *Pseudomonas hydrophila*. It is not at all surprising to note that *Pseudomonas hydrophila* was equally well enriched by glucose and starch media, when we take into account its ability to produce acid and gas from the same media. But it is a matter of surprise to find *Pseudomonas astragali*, which does not produce acid or gas from either of these two media, growing in the same. From this, it would appear that it is the association of these two organisms, that has brought about the abovementioned results. Formate and succinate appear to serve as specific enrichment for *Pseudomonas æruginosa*. *Pseudomonas æruginosa* and *Pseudomonas arvilla* are stimulated by the use of lactate and malate. From the above, it is evident, that but for the enrichment of *Pseudomonas arvilla*, we have at our disposal suitable substrates

TABLE III

Bacterial Enrichment of the Flora associated within the Galls of Pongamia glabra *Vent.**

Carbon Source	Specific Suitability for Isolation of				
	Ps. æruginosa	*Ps. arvilla*	*Ps. astragali*	*Ps. hydrophila*	*M. citreus*
1 Sodium oxalate ..	−	−	+	−	−
2 Sodium formate .	+	−	−	−	−
3 Sodium lactate ..	+	+	−	−	−
4 Sodium malate ..	+	+	−	−	−
5 Sodium fumarate ..	−	−	−	+	−
6 Sodium succinate ..	+	−	−	−	−
7 Sodium citrate ..	−	−	−	+	−
8 Glucose ..	−	−	+	+	−
9 Starch ..	−	−	+	+	−

Legend : * In the absence of organic nitrogen
+ Suitable
− Unsuitable

for specific stimulation and isolation of the remaining 3 *Pseudomonas* species from this natural habitat, even though all the 4 may exist in a confusing mixture.

Coming now to other inclusions in the galls, we observed only the larval forms of *Aspondylia pongami* F. The reason for not finding the subsequent forms, may be due to the fact, that they have been found to be mortally affected by a large number of mites (tetrapod species of *Eriophyes*), which were observed to be infesting the trichome-like vegetable hairs inside the gall and also seen emerging out of the larvæ, after rupturing the cuticle of the same.

DISCUSSION

From the above experimental results, several interesting points emerge out. Firstly, it appears that the microflora present in the leaf-galls of *Pongamia glabra* Vent., comprises primarily of *Pseudomonas* and secondarily of a *Micrococcus*, well known for its ubiquity and presence as a secondary invader in several animal infections. Because of its ubiquitous nature,

it can be discarded as of minor importance from the point of view of gall-formation. Of the four *Pseudomanas* encountered, three are of proved pathogenicity and hence need no special reference here. As a matter of fact, Clara's pathogen for tobacco (1930) has been pointed out by Braun and Elrod (1942) as *Pseudomonas æruginosa* and the only species described in the literature as *Pseudomonas astragali*, is so named because of its phyto-pathogenic action on plant species of *Astragalus* genus: *Pseudomonas hydrophila* has also been pointed out as pathogenic to fish, frog and other water animals, and therefore it may be reasonable to assume that all these species, together with the insects associated (found in the galls), constitute the causative factors in the gall-formation and that it is quite improbable that insects alone are the ætiological agents. It should also be remembered that the *Pseudomonas* is the most important genus embracing plant pathogenic bacteria, and most of the species isolated during this study are mentioned as pathogenic in character. Moreover, the pathogenicity of the strains of *Ps. æruginosa* isolated have been proved by mouse inoculation tests carried out subsequently. Now if their pathogenic character is viewed in the light of the habitat from which they were isolated, it becomes increasingly difficult not to associate them from having pathogenic significance in the formation of leaf-galls on *Pongamia glabra* Vent.

Summary

Microbiological and entomological findings made from the studies on the leaf-galls of *Pongamia glabra* Vent., are presented. Use of various routine and enrichment media resulted in the isolation of *Pseudomonas æruginosa*, *Pseudomonas arvilla*, *Pseudomonas astragali*, *Pseudomonas Hydrophila* and *Micrococcus citreus*. In the light of the revelations made, a brief discussion has been he d, and it has been suggested that the insects as well as the bacteria associated may symbiotically be the cause for the galls to form and that the insects by themselves may not be the sole factor responsible.

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PALM AND FINGER PRINTS OF CONJOINED TWINS OF BANGALORE

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This paper is a continuation of the study by the authors (1950) of the conjoined twins of Bangalore (Fig. 1). The palm prints were taken in 1950 at the residence of the twins. Though the prints obtained were reasonably good there were some areas where the impression was blurred and difficult of interpretation. We were hoping we might get a chance to obtain more ideal impressions. But unfortunately the conjoined twins subsequently got ill and passed away in February 1952. The palm prints here presented are the only ones ever taken of these conjoined twins that are available for scientific study.

Introduction

There is a fundamental symmetry obvious in the external conformation of any vertebrate animal necessitated by the demands for its locomotion. The two halves of the body though apparently symmetrical, yet reveal on scrutiny, certain asymmetries in features pertaining to integument, viscera, etc. Among the various features of asymmetry or asymmetry reversal (mirror imaging) studied in twins are, the palmar dermatoglyphs and finger prints. A study of these would reveal the play of asymmetry mechanism or its reversal in mono-zygotic twinning where both the individuals inherit the identical genetic make up. In the case of monozygotic separated twins, whatever may be the criteria by which we diagnose them as identical, there is always the remote possibility of the twins being dizygotic showing very closely resembling characters as siblings some times do. But in conjoined twins we get two individuals that are absolutely identical in their genetic make up having been derived from the same zygote and the study of their palm prints will establish the way in which the *asymmetry mechanism or its reversal plays in individuals of unimpeachable genetic identity*. Hence the study of palm prints of conjoined twins is of great value as they are extremely rare.

Method of Obtaining Palm Prints

The palm prints were obtained by the standard technique described by Cummins *et al.* (1929) in which printer's ink evenly spread on a glass

plate by a roller is applied over the palm, and prints obtained on glazed paper.

FORMULATION OF MAIN LINES AND PATTERNS (GENERAL)

The interpretation of a palm print and its formulation are made on certain criteria of identification of digital triradii, palmar main lines, axial triradii and patterns in accordance with accepted conventional standards enunciated by previous workers (Cummins *et al.*, 1929) to which reference should be made. However, a brief account of the methodology of palm print interpretation is given hereunder to enable the reader not familiar with the technique to follow the formulation and interpretation of the palm prints in this study.

A triradius is a locus where ridges from three directions seem to meet. There are four triradii in the palm proximal to the bases of the fingers which are called the digital triradii. These are conventionally named as digital triradii—*a*, *b*, *c* and *d* from the radial to the ulnar side (Fig. 2).

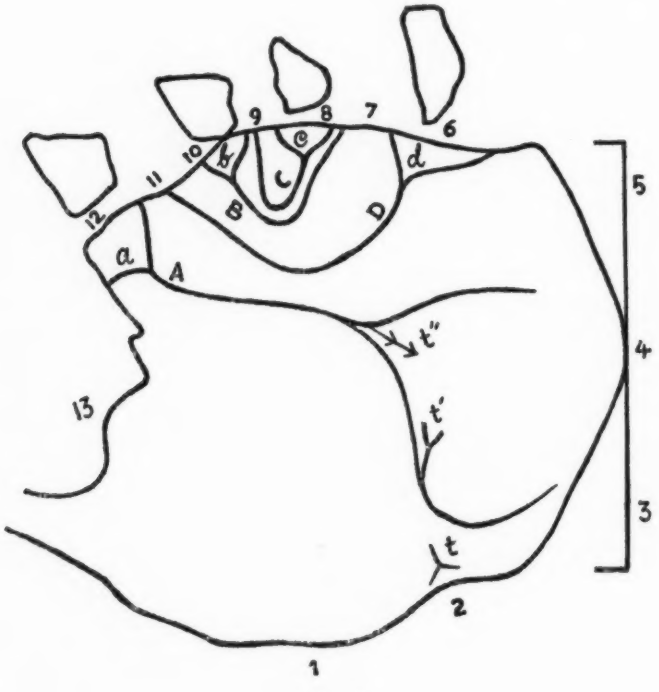

FIG. 2

The proximal radiants of the digital triradii are called the palmar main lines and these after a varying course across the palm terminate along the border of the palm. These main lines are designated as *A*, *B*, *C* and *D* in radio-ulnar sequence, using capital letters to distinguish them from the digital triradii which are symbolised by the small letters *a*, *b*, *c* and *d*. To describe the termination of main lines, different areas along the border of the palm are arbitrarily indicated by numbers 1 to 13 as shown in Fig. 2. To formulate the main lines, their terminations alone are indicated.

Along the long axis of the palm, *i.e.*, approximately along a line joining the base of the ring finger to the mid point of the proximal border of the palm, there occur certain triradii called axial triradii at different levels and these are designated by symbols *t*, *t'* and *t''* (Fig. 2) for proximal, intermediate and central triradii.

Further, there are certain ridge configurations in the palm called patterns. The full set of palm patterns are six, consisting of hypothenar, thenar and first interdigital (often associated), second, third and fourth interdigital patterns. These patterns may be in the form of whorls, loops, arches and tented arches, which are indicated by the symbols—*W*, *L*, *A* and *T* respectively and the exponent *u*, *r* and *c* are used to indicate the direction in which they open, ulnar ward, radial ward and carpal ward respectively. An absence of a pattern when the lines run as parallel ridges is an open field and is designated by symbol 0.

The palmar formula is a complete description of the palm print consisting in order, of palmar main lines, axial triradii and palm patterns. The main lines are formulated by their symbols in the sequence of line *D*, *C*, *B* and A and the symbols are separated by periods. This part of the formula is punctuated by a dash and the symbols for the axial triradii follow, which is again separated by a dash from the palm patterns designated by their symbols in order—hypothenar, thenar and first interdigital, second, third and fourth interdigital patterns, each separated by a period. When two main lines course closely separated by not more than two ridges, the possibility of a different interpretation is shown in the formulation by the symbol of the line actually traced succeeded by the symbol of the line closely followed in brackets as *e.g.*, 7 (8) in Fig. 3, which is called an alternative formulation. In instances where the axial triradius is indeterminable due to defective printing it is indicated by the symbol " ? ".

Finger Prints

The configurations usually formed by the ridges on the skin of the volar aspects of the digits corresponding to the terminal phalanges are the finger

print patterns. These may be whorls, loops, tented arches and arches. Rarely we get open fields on finger prints.

FORMULATION OF MAIN LINES AND PATTERNS

The palmar formulæ of the conjoined twins Gouri and Ganga are as follows:—

	Right	Left
Gouri	7 (8).5″ (6).5″.4–*t*′–A^{u}.0.0.0.L^{u}	7·5″.5″.4–*t*′–A^{u}.0.0.0.L^{u}
Ganga	7 (8).5″ (6).5″.4–?–A^{u}–0.0.0.L^{u}	7·5″.5″.4–*t*′–A^{u}.0.0.0.L^{u}

PECULIARITIES OF MAIN LINES AND PATTERNS

Line A.—Its termination is at 4 in all the palms; yet it is more distalwards in the right hand of Gouri and left hand of Ganga suggestive of an attempt at mirror-imaging.

Line B.—Its termination is at 5″. Though the same value is given for its termination in all the palms there is a shifting of the termination distalwards from the distal transverse crease of the wrist in the right hands of both and to a very slight extent in the left hand of Gouri indicating normal asymmetry.

Line C.—Its termination is at 5″. Though the same value is given for its termination in all the palms, in the left hands of both it is more proximal whereas in the right hands of both it is more distal, showing normal asymmetry.

Line D.—In all the palms this line terminates at 7. Its termination in both right hands tends towards the base of the ring finger whereas in the left hand it is in the fourth interdigital space. Lines C and D in both right hands closely follow each other with one or two minute ridges in between and hence each line gets an alternative formulation.

Patterns.—The hypothenar pattern as a simple arch is present in all the palms. All the palms have the fourth interdigital pattern represented as a loop.

Finger Prints.—The finger prints show patterns as given below.

	Right Hand					Left Hand				
	I	II	III	IV	V digits	I	II	III	IV	V digits
Gouri	U	W^{u}.	U.	W^{u}.	U.	A.	Wl?	U.	U.	U.
Ganga	U	W^{u}.	U.	W?.	U.	W^{u}.	U.	Wl?.	U.	U.

U. = Ulnar Loop.

W^u. = Ulnar Whorl.

?. = Direction indeterminable.

A. = Flat Arch.

Wl. = Whorl within a loop.

Discussion

In anthropoid apes and primitive races, it has been observed (Wilder, cited by Newman, 1930) that the main lines have a tendency to run more or less in a parallel manner inclining proximally. With advancing evolution the lines shift their course to run more obliquely terminating near the distal border of the palm. Further, in normal right-handed individuals the right hands are more advanced and the left hands are comparatively more primitive. It has also been noted that in left-handed individuals this asymmetry is reversed. In any individual the more skilled hand, irrespective whether it be right or left, indicates its more advanced feature by a higher total of the numbers of its formula of palmar lines. The dextrous hand shows a shifting of the termination of the main lines more distally and radially as contrasted with the less dextrous hand showing terminations more proximally and ulnarwards. This general tendency of the advanced condition of the more dextrous hand and the primitive condition of the less dextrous hand has been recognized and Newman (1930) designates the appropriate formulæ of the main lines as radial and ulnar formulæ respectively. The former occur nearly twice as frequently on right hands as compared with left hands and *vice versa*.

The formula of the main lines for all the four palms shows a primitive condition for the lines individually and also for the summated value, implying that all the four palms show an ulnar formula indicating non-dextrality. This seems to be a significant and noteworthy feature requiring an explanation. Among other characters also pointing to the primitive nature of the hands are the presence of the 4th interdigital pattern and a hypothenar pattern in all the four palms and the relatively large number of whorls (7 out of 20) in the finger prints.

As to how the four hands of the twins are nearly identical in palmar dermatoglyphs and all are non-dextrous is to be explained by a consideration of the play of asymmetry mechanism. The hypothetical alternatives can be postulated as follows:—

If the original zygote from which the conjoined twins were derived, was genetically destined to form a right-handed individual and the splitting of the embryo took place to form the conjoined twins, we should expect to have one twin showing the normal asymmetry (right-handed) and the other showing either normal asymmetry (right-handed) if twinning was early or a reversed asymmetry (left-handed) if twinning was late. This does not however agree with the condition found in the hands of these conjoined twins.

If the original zygote was genetically destined to form a left-handed individual and the splitting took place to form the conjoined twins, we should have one twin showing left-handed asymmetry and the other showing the same asymmetry or its mirror-image, depending on whether twinning happened early or late respectively. In either case, one of the hands of each twin will be a dextrous hand (showing a radial formula) and the other hand a non-dextrous one (showing an ulnar formula).

Both the alternatives considered above are not applicable to the set of conditions found in the palms of the conjoined twins.

Hence the suggestion is put forward that these twins were obtained from an original zygote that probably gave rise to triplets by a process of

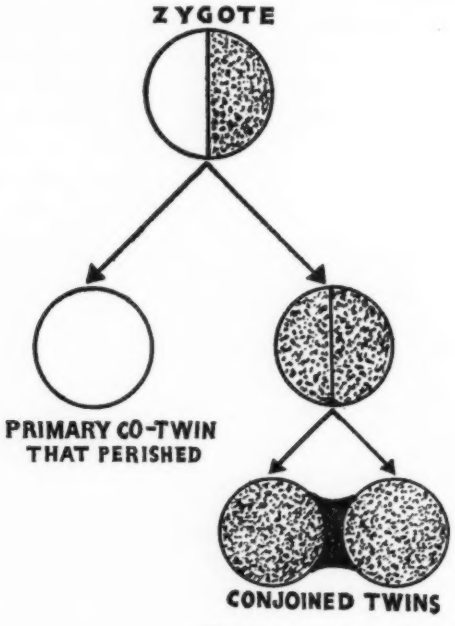

FIG. 5

super-twinning. The conjoined twins are derived by a secondary twinning of the twin from the inferior side of the original zygote after an initial twinning, while the co-twin derived from the superior side has somehow perished (Fig. 5). The primary and secondary splittings are supposed to have taken place rather late in development, after the normal asymmetry mechanism had indicated the bias towards the superior and inferior side of the halves of original embryonic area. Hence the secondary twinning of the non-dextrous or inferior side gave rise to twins without a strongly expressed asymmetry mechanism.

Summary

All vertebrates though they show a basic bilateral symmetry in their external conformation, yet reveal on scrutiny certain asymmetric characters of integument, viscera, etc. Monozygotic twins reveal the play of asymmetry mechanism or its reversal (mirror imaging) in such characters. A study of this mechanism in conjoined twins affords criteria for judging individuals of identical genetic make up.

The palm and finger prints are interpreted on certain accepted methods enunciated by previous workers (Cummins *et al.*, 1929 and Newman, 1930). Palm prints are interpreted by taking into consideration palmar main lines, axial triradii and palm patterns, all of which are symbolised and reduced to a formula. In anthropoid apes and primitive races it has been established (Wilder, cited by Newman, 1930) that the palmar main lines run more or less in a parallel manner inclining proximally. With advancing evolution, the lines shift their course and run more obliquely and terminate near the distal border of the palm. In right-handed individuals, the right hands show the more advanced condition of the palmar lines and the left hands show the primitive condition. This asymmetry is reversed in left-handed individuals. The dextrous hand, whether it be right or left, shows the advanced condition of the palmar main lines shifting their terminations distally and radialwards and the less dextrous hand showing the terminations more proximally and ulnarwards; and such main line formulæ are designated radial and ulnar formulæ respectively.

All the four hands of the conjoined twins show ulnar formulæ. Also the presence of hypothenar pattern and fourth interdigital pattern indicate a primitive or less dextrous nature. Their finger prints also show a preponderance of primitive patterns—7 whorls out of 20.

The conjoined twins possess in the four hands nearly identical palmar dermatoglyphs indicating non-dextrous nature, without exhibiting normal

asymmetry. To explain this condition the suggestion is put forward that the original zygote gave rise to triplets by a process of super-twinning, the conjoined twins being derived by secondary twinning of the twin from the inferior side, while the co-twin from the superior side had perished.

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FIG. 1

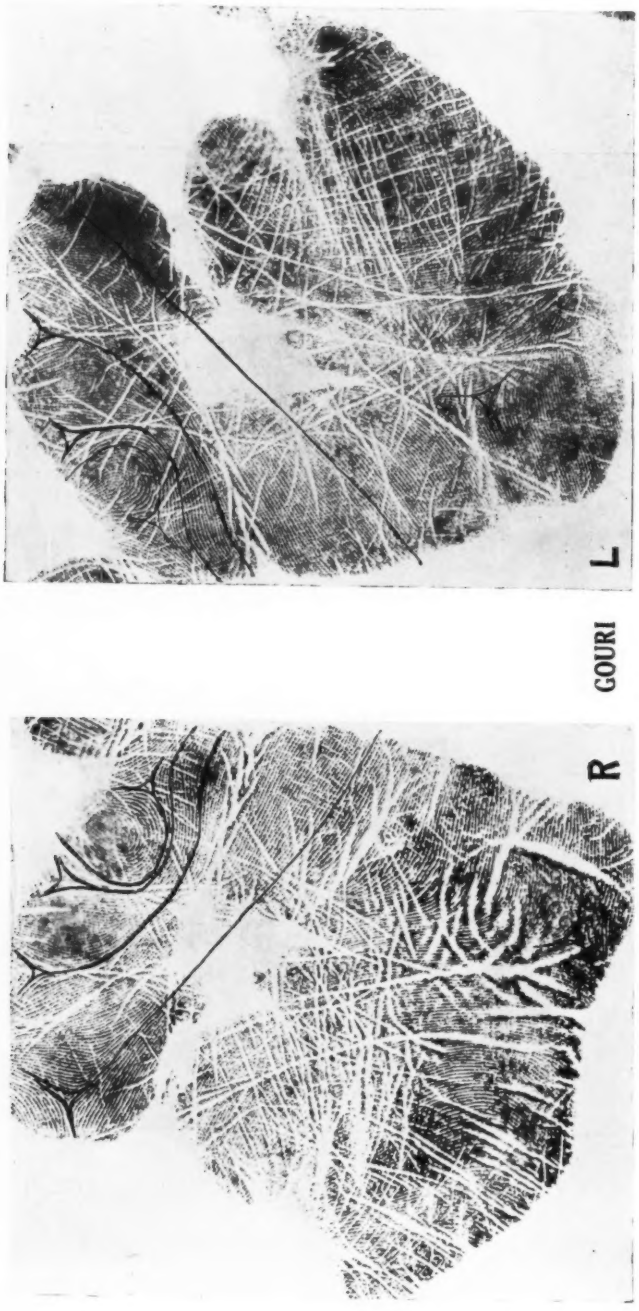

FIG. 3

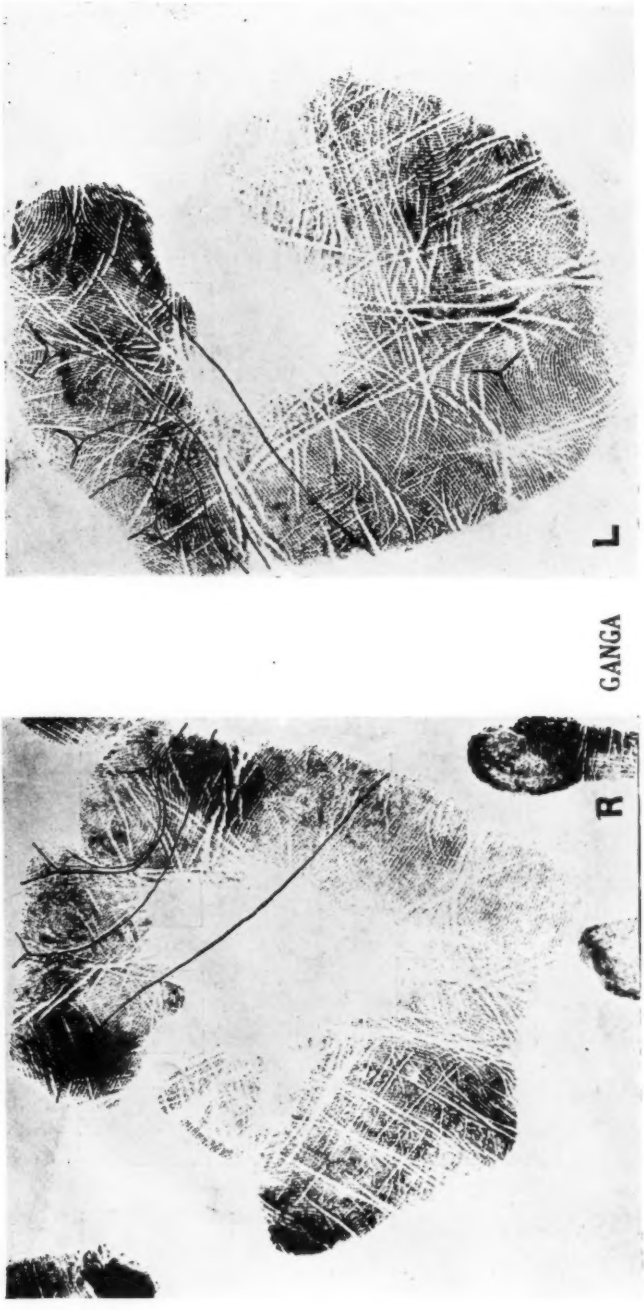

FIG. 3

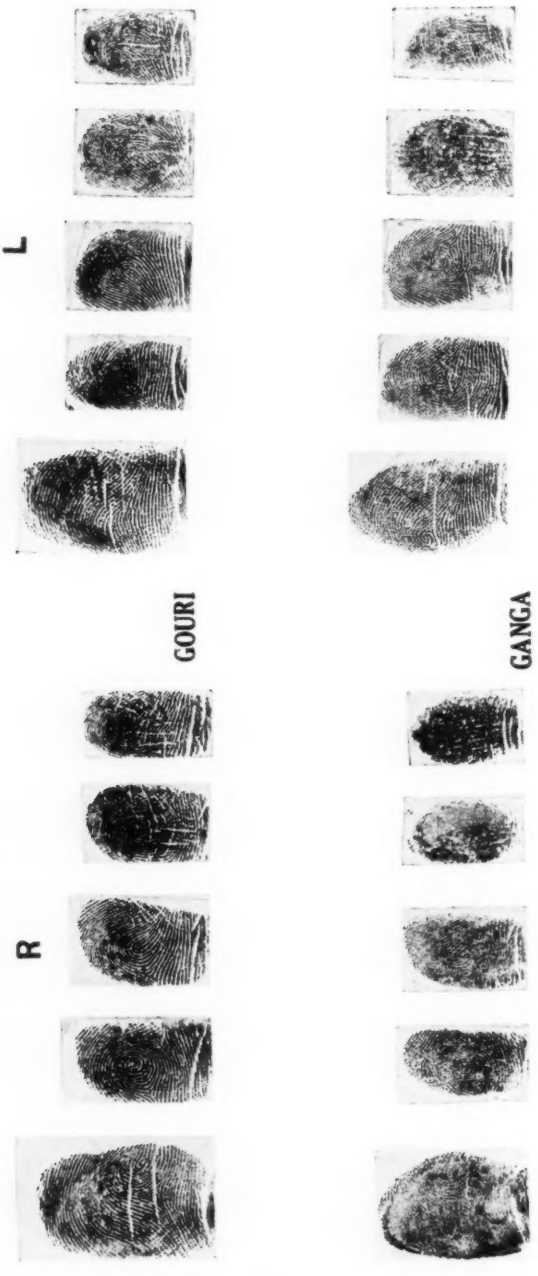

FIG. 4

ADDITIONS TO THE FUNGI OF MADRAS—XV

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Synchytrium emiliæ sp. nov.

Galls numerous, on petioles and lamina, minute, crowded, yellow to brown, amphigenous; hypnospores spherical, solitary or sometimes two in each gall, dark brown, 47–124 μ, wall thick, 3-layered, epispore dark brown, up to 15 μ thick; sporangial sorus yellow, subglobose or oval, 78–140 $\times$ 65–93 μ, made up of numerous sporangia; sporangia rounded or angular by pressure, thin walled, 19 $\times$ 15 μ (16–22 $\times$ 12–19), yellow.

Gallæ plures, in petiolis atque foliis, minutæ, aggregatæ, luteæ vel brunneæ, amphigenæ; hypnosporæ sphæricæ, solitariæ vel nonnumquam binæ in singulis gallis, fusce brunneæ, 47–124 μ, parietibus crassis, 3-seriatis, episporio fusce brunneo, usque ad 15 μ crasso. Sorus sporangialis luteus, subglobosus vel ovalis, 78–140 $\times$ 65–93 μ, constans ex plurimis sporangiis; sporangia rotundata vel angularia ob pressionem, tenuibus parietibus prædita, 19 $\times$ 15 μ (16–22 $\times$ 12–19), lutea.

On petiole and lamina of *Emilia sonchifolia* DC. (Compositæ), Bantwal (South Kanara), 27-8-52, T. S. Ramakrishnan.

The infected portions are thickened and the galls form a brownish crust on both sides of the leaves. The resting spores which are formed freely, are thick walled. Outside the epispore is an indefinite yellowish layer. The sorus escapes out of the resting spore as a yellowish globular body with numerous sporangia. The fungus bears a resemblance to *S. vulgatum* Rytz. But comparison with an authentic specimen of *S. vulgatum* however showed that the fungus under study is different as judged by the size, appearance and formation of the hypnospores.

Meliola stenospora Winter

Saccardo, P. A., *Syll. Fung.*, **9**, 423, 1891.

On living leaves of *Strychnos nuxvomica* L. (Loganiaceæ), Taliparamba (Malabar), 27-7-52, N. V. Sundaram.

The fungus is prevalent in several parts of Malabar and South Kanara. The colonies form black growths mostly epiphyllously. Hyphopodia are alternate or sometimes opposite with subglobose to cylindrical top cells, measuring up to 18–24×9–12 μ. Very rarely pointed hyphopodia are also noticed. The hyphæ are up to 9 μ thick and bear numerous black, long, multicellular pointed setæ up to 350 μ long. The perithecia are globose sometimes depressed at the top and have rugose surface. They are 62–127 μ in diameter. The spores are 4 septate, dark brown, 34×12 μ (28–40×9–16), constricted at the septa and with rounded ends.

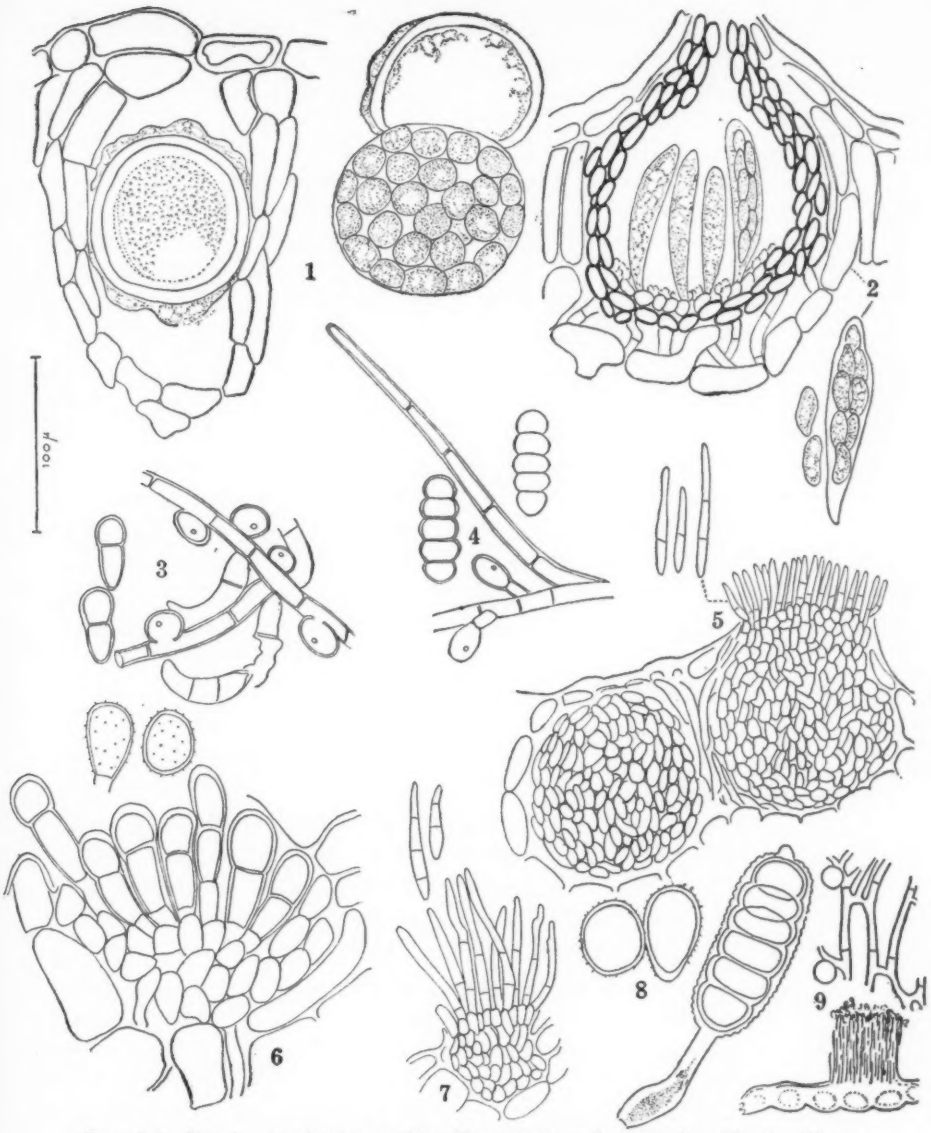
Schiffnerula malabarensis sp nov.

Colonies amphigenous, thin, black; mycelium of brown hyphæ forming loose network, up to 9 μ thick; hyphopodia alternate or sometimes opposite, sessile, subglobose, one celled, sometimes elongate and tapering, measuring 12–18×9–12 μ, the longer ones two celled; perithecia globose, scattered, 78–170×69–150 μ; ascospores dark brown, ellipsoid, one septate, constricted at the septum, the upper cell shorter than the lower cell, 12·6×5·6 μ (11–16×4–7).

Coloniæ amphigenæ, tenues, nigræ; mycelium constans hyphis brunneis efformantibus reticulationem quamdam liberam, ad 9 μ crassum; hyphopodia alterna vel aliquando opposita, sessilia, subglobosa, unicellulata, aliquando elongata atque fastigata, magnit 12–18×9–12 μ, longiora quæque bicellulata; perithecia globosa, dispersa, 78–170×69–150 μ; ascosporæ fusce brunneæ, ellipsoidæ semel septatæ, constrictæ ad septum, cellula superiore breviore quam inferiore, 12·6×5·6 μ (11–16×4–7).

On living leaves of *Passiflora edulis* Sims. (Passifloraceæ), Wynaad, 10–8–52, K. Govindakutty Kurup.

The fungus forms a sooty growth on both sides of the leaves. The hyphæ penetrating through the stomata can be seen here and there. Otherwise the mycelium is superficial. The perithecia are superficial and occur as black round bodies distributed on the sooty growth. By the time the perithecia are mature the ascus is not clearly distinguishable. The spores come out in groups of 6 to 8 when the perithecium is crushed. The two-celled nature of the ascospores and the presence of hyphopodia show that the fungus is to be included in *Schiffnerula*. A prominent pore is seen in the hyphopodial cell. *S. mirabilis* v. Höhn has been recorded on leaves of *Passiflora* sp. from Java; but the measurements of the perithecia and the ascospores are widely differing. Perithecia of the fungus under study are more than twice as big and the ascospores are much smaller than those of



FIGS. 1–9. Fig. 1. *Synchytrium emiliæ* – Hypnospore and sporangia. Fig. 2. *Phomatospora artocarpi*—Section through perithecium and an ascus. Fig. 3. *Schiffnerula malabarensis*—Hyphopodia and ascospores. Fig. 4. *Meliola stenospora*—Hyphopodia, seta and ascospores. Fig. 5. *Ramulispora capparidis*—Section showing sclerotioid stromata, conidiophores and conidia. Fig. 6. Telium of *Puccinia curcumæ* and urediospores. Fig. 7. *Cercospora wendlandiæ*—conidiophores and conidia. Fig. 8. Urediospores and teliospore of *Phragmidium butleri*. Fig. 9. *Beniowskia graminis* – Sporodochium (diagrammatic) and hyphæ with conidia.

S. mirabilis. Furthermore the ascospores of the latter are described as hyaline whereas they are dark brown in the former.

Phomatospora artocarpi sp. nov.

Spots irregular, of varying sizes, grey coloured with a dark brown margin; perithecia mostly epiphyllous, subepidermal, appearing as minute black dots, ostiolate, 130–170×126–155 μ; peridium of 2–5 layers of dark brown cells; asci cylindrical, clavate, hyaline, thin-walled, 78×12 μ (59–96×9–16); aparaphysate; ascospores 8, one-celled, elliptic, sometimes slightly curved, with rounded ends, irregularly biseriate, hyaline, 15·5×6 μ (12–19×4–8).

Maculæ irregulares, magnitudinis diversæ, griseæ sed marginibus fusce brunneis; perithecia ut plurimum epiphylla, subepidermalia, apparenthia ut minuta puncta nigra, ostiolata, 130–170×126–155 μ; peridium constans 2–5 seriebus cellularum fusce brunnearum; asci cylindrici, clavati, hyalini, parietibus tenuibus præditi, 78×12 μ (59–96×9–16), aparaphysati; ascosporæ 8, uni-cellulatæ, ellipticæ, nonnumquam tenuiter curvatæ, apicibus rotundatis, irregulariter biseriatæ, hyaline, 15·5×6 μ (12–19×4–8).

On living leaves of *Artocarpus hirsuta* Lamk. (Moraceæ), Gudalur (Nilgiris), 25–5–52, N. V. Sundaram.

The spots vary in size according to the place of formation.

Aecidium gymnematis sp. nov.

Rust spots yellowish, indefinite, almost circular; pycnia mostly hypophyllous, subepidermal, globose, 196–280×169–210 μ; æcia hypophyllous, clustered, cupulate, peridiate, margin of the peridium lacerated and reflexed, 280–490×210–310 μ; peridial cells polygonal, 28–47×16–31 μ, hyaline, the free surfaces prominently verrucose; æciospores subglobose, catenulate, 28×25 μ (25–35×21–28), wall irregularly thickened, finely verrucose, contents yellowish orange in colour.

Maculæ luteolæ, indefinitæ, fere circulares, ut plurimum constantes ex pycniis hypophyllæ, subepidermales, globose, 196–280×169–210 μ; æcia hypophylla, aggregata, cupulata, peridiata, marginibus laceratis atque reflexis, 280–490×210–310 μ; cellulæ peridiales polygonales, 28–47×16–31 μ, hyalinæ, verrucosæ in faciebus liberis; æciosporæ sub-globosæ, catenulatæ, 28×25 μ (25–35×21–28), parietibus irregulariter incrassatis, minute verrucosis, contentis aurantiacis colore.

On living leaves of *Gymnema sylvestre* R. Br. (Asclepiadaceæ), Manantoddy (Malabar), 26–5–52, N. V. Sundaram.

The rust occurs in indefinite spots on the leaves. The upper surface is yellowish. On the lower surfaces the æcia and pycnia are mixed together in crowded groups. The pycnia are reddish brown and open out flush with the epidermis. The æcial cups have a whitish reflexed peridium with orange coloured mass of spores inside.

Aecidium walayarense sp. nov.

Spots rounded, amphigenous, hypertrophied, convex on the lower surface and concave on the upper surface, brownish; pycnia epiphyllous, subepidermal, 140–210 × 126–182 μ, reddish brown, paraphyses projecting beyond the epidermis in mass; æcia crowded and immersed in the thickened tissue, hypophyllous, 330–518 × 210–330 μ, peridiate, peridial cells polygonal, 21–35 × 15–25 μ; æciospores subglobose, catenulate, 22 × 19 μ (19–25 × 18–22), wall uniform, finely verruculose, contents yellowish.

Maculæ rotundatæ, amphigenæ, hypertrophiatæ, convexæ in inferiore atque concavæ in superiore superficie, brunneolæ; pycnia epiphylla, subepidermalia, 140–210 × 126–182 μ, rubrobrunnea; paraphyses emergentes ex epidermate in massa; æcia aggregata atque immersa in textura crassa, hypophylla, 330–518 × 210–330 μ, peridiata, cellulæ peridiales polygonales, 21–35 × 15–25 μ; æciosporæ subglobosæ, catenulatæ, 22 × 19 μ (19–25 × 18–22), parietibus uniformibus, minute verruculosis, contentis luteolis.

On living leaves of *Cordia obliqua* Willd. (Boraginaceæ), Walayar (Malabar), 12–6–52, N. V. Sundaram.

The infected portion is thickened. A hemispherical swelling is formed on the lower surface of the leaf and the æcia are arranged on this. There is a corresponding depression on the upper surface. The pycnia have no definite shape. They are formed subepidermally but occupy a position between the palisade and the epidermis. The æcia are deep-seated.

Three species of *Aecidium*, *viz.*, *A. brasilense* Diet., *A. cordiæ* P. Henn. and *A. lindavianum* Syd., have been recorded on this host genus from America. But the rust under study differs from them in the position of the pycnia, the formation of galls and in spore size.

Phragmidium butleri Sydow.

Sydow, H. and P. and Butler, E. J., *Ann. Mycol.*, **5**, 501, 1907.

On living leaves of *Rosa leschenaultiana* W. and A. (Rosaceæ), Ootacamund, 20–5–52, N. V. Sundaram.

Uredia and telia are present. The former are orange coloured and are surrounded by numerous hyaline incurved paraphyses. Telia appear as

black growths. The pedicel of the teliospore is hyaline, up to 68 μ long, hygroscopic and swelling gradually at the base. The urediospores measure 26 × 22 μ (22–31 × 19–25). The teliospore is 3–7 septate, reddish brown in colour, 78 × 28 μ (50–91 × 25–31), prominently verrucose and with a short hyaline apiculus.

Puccinia curcumæ sp. nov.

Uredia hypophyllous, minute, subepidermal, light yellow; urediospores subglobose to ovate, with very short stalks, echinulate, 31 × 24 μ (25–47 × 19–28); telia hypophyllous, light yellow, subepidermal, erumpent, minute; teliospores pedicellate, pedicel up to 12 μ long, two-celled, clavate, apex slightly thickened, up to 6 μ, constricted at the septum, 37 × 16 μ (28–47 × 12–19), either pointed or rounded at the apex, subhyaline, germinating *in situ*.

Uredia hypophylla, minuta, subepidermalia, pallide lutea; uredosporæ subglobosæ vel ovatæ, pedicellis brevissimis, echinulatæ, 31 × 24 μ (25–47 × 19–28); telia hypophylla, pallide lutea, subepidermalia, erumpentia, minuta; teliosporæ pedicellatæ, pediculo usque ad 12 μ longo, 2-cellulatæ, clavatæ, apice tenuiter crasso usque 6 μ, constrictæ ad septum, 37 × 16 μ (28–47 × 12–19), vel acutæ vel rotundatæ ad apicem, subhyalinæ, germinantes *in situ*.

On living leaves of *Curcuma* sp. (Zingiberaceæ), Mundage (South Kanara), 27–8–52, T. S. Ramakrishnan.

The rust forms minute, dot-like sori clearly visible on the lower surface. It bears a resemblance to the rust, *Uredo amomi* Petch, recorded on *Amomum* sp. But both telia and uredia are present on *Curcuma*. Inoculations were carried out with viable urediospores on *Canna indica*, *Maranta arundinacea* and *Curcuma longa*. Successful infection was obtained on *C. longa* alone.

Cercospora wendlandiæ sp. nov.

Spots irregular, amphigenous, brownish grey on the upper surface and brown on the lower surface; conidiophores developed on sub-stomatal erumpent stromata, amphigenous, black in mass; individual conidiophores smoky brown, straight or bent, sparsely septate, 24–75 × 3–4·5 μ; conidia subhyaline, elongated, tapering towards the apex, septate up to 5 septa, 28–46 × 4·5–6 μ.

Maculæ irregulares, amphigenæ, brunneolo-griseæ in superiore, brunneæ vero in inferiore superficie; conidiophori insidentes stromatibus erumpentibus substomatalibus, amphigeni, nigri in massa; singuli conidiophori

effumato-brunnei, recti vel curvati, sparse septati, 24–75×3–4·5 μ; conidia subhyalina, elongata, fastigata ad apicem, usque quinquies septata, 28–46×4·5–6 μ.

On living leaves of *Wendlandia notoniana* Wall. (Rubiaceæ), Manantoddy (Wynaad), 26–5–52, N. V. Sundaram.

Irregular spots are formed on the leaves. The fructifications appear as black dots on the upper surface. Well-developed stromata are present.

Ramularia mimosæ Stev. and Dalbey

Stevens, F. L. and Dalbey, N. E. *Mycologia*, **11**, 6, 1919.

On *Mimosa pudica* L. (Mimosoideæ), Kallar (Coimbatore), 15–5–52, N. V. Sundaram.

The fungus forms white patches and resembles in appearance growths of powdery mildew. The conidiophores are hyaline and arise in clusters emerging through the stomata. They measure 12×4·5 μ (9–19×3–8). The conidia are hyaline and tapering and measure 25×3 μ (13–40×1·5–3·5).

Beniowskia graminis Rac.

Raciborski, M. *Parasit. Algen und Pilze*, Javas, II, 37, 1900.

On living leaves of *Pennisetum hohenackeri* Hochst. (Gramineæ), Wynaad (Malabar), 27–5–52, N. V. Sundaram.

The sporodochia are white and erumpent with a mealy appearance. The hyphæ form a compact column bursting through the epidermis. At the top they form a loose anastomosing mass. At the free end the hyphæ are hyaline, septate, often twisted or coiled. Conidia are borne laterally. These are globose, hyaline one celled and measure 10·5 μ (6·5–12) in diameter.

Glæosporium alborubrum Petch

Petch, T., *Ann. Roy. Bot. Gard. Peradeniya*, **3**, 8, 1906.

On living leaves of *Hevea brasiliensis* M. Arg. (Euphorbiaceæ), Koney (Travancore State), 5–6–52, Superintendent, Koney Estate.

The fungus causes die back of the twigs and defoliation of the young plants.

Ramulispora capparidis sp. nov.

Spots amphigenous, circular, brown with a well-defined raised margin; conidiophores borne on prominent hypophyllous dark brown sclerotioid

stromata, 62–99×46–76 μ, septate, subhyaline, branching, 24–46×1·5–3 μ; conidia linear, hyaline, 1–4 septate, 12–43×1·5–2·5 μ.

Maculæ amphigenæ, circulares, brunneæ, marginibus bene definitis elevatis; conidiophori insidentes stromatibus fusce brunneis prominentibus hypophyllis sclerotioideis, 62–99×46–76 μ, septati, subhyalini, ramosi, 24–46×1·5–3 μ; conidia linearia, hyalina, 1–4 septata, 12–43×1·5–2·5 μ.

On living leaves of *Capparis grandiflora* Wall. (Capparidaceæ), Maruthamalai (Coimbatore), 27–6–52, N. V. Sundaram.

Distinct spots are formed on the leaves. The fructifications appear as whitish dots on the lower surface. Black sclerotioid subepidermal bodies are formed. Later conidiophores are developed on these. The sclerotioid stromata indicate that the fungus should be included in the genus *Ramulispora*. Some of the sclerotioid bodies are round and do not bear conidiophores.

Macrophoma toddaliæ sp. nov.

Spots irregularly circular, amphigenous, greyish white on the upper surface, zonate, pycnidia mostly epiphyllous, sometimes hypophyllous, innate, erumpent, black, often arranged in rings; pycnidiospores oblong, hyaline, one celled, 11·2×5·6 μ (8–14×4–7), with granular contents.

Maculæ irregulariter circulares, amphigenæ, griseo-albidæ in superiore facie, zonatæ; pycnidia ut plurimum epiphylla, aliquando hypophylla, innata, erumpentia, nigra, sæpe annulariter disposita; pycnidiosporæ oblongæ, hyalinæ, unicellulatæ, 11·2×5·6 μ (8–14×4–7), contentis granularibus.

On living leaves of *Toddalia asiatica* Lamk. (Rutaceæ), Ootacamund, 20–5–52, N. V. Sundaram.

The spots were of varying sizes, but very characteristic with several concentric zones. Each zone is bounded by a greyish brown ring. The colour of the spot varies according to age.

We are indebted to Dr. H. Santapau of St. Xavier's College, Bombay, for the Latin translations. Our thanks are due to the Government Lecturing and Systematic Botanist, Coimbatore, for identifying some of the host plants.

TERTIARY SPORES AND POLLEN FROM WARKALLI LIGNITES, TRAVANCORE

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[Communicated by Prof. L. Narayana Rao, F.A.Sc., M.Sc., Ph.D. (Lond.), F.L.S.]

Introduction, Material and Method

The discovery of a large number of spores and pollen and other microfossils in Palana and Dandot lignites (Rao and Vimal, 1952; Vimal, 1952) encouraged me to study the lignites of the other Tertiary deposits of India.

Through the kind courtesy of the Secretary, Industries Section, Development Department of the Travancore-Cochin Government, some samples of lignite from Warkalli were obtained. These samples, on maceration, yielded a large number of microfossils, like spores, pollen and cuticles. Some of these have already been described, compared and illustrated in a preliminary paper (Rao and Vimal, 1952). The present paper deals with the types recovered after further examination of the material. The sporomorphs have been numbered in continuation of those described in the previous paper.

The material was allowed to remain in Nitric acid for about two days. It was then repeatedly washed in distilled water, and subsequently treated with 10% sol of KOH for about two hours. As the material still showed a lot of silica remains, like sand grains, some HF acid was added to it and heated over a sand-bath for about an hour. All this process was done inside a fume chamber. Finally the material was washed and put in glycerine. Permanent slides were made in pure glycerine and sealed with Gold size. All possible precautions have been taken to avoid laboratory and atmospheric contaminations. As the permanent mounts were made in glycerine, it was possible to study both the equatorial and polar views of the grains by making them roll over, and they have been sketched accordingly. The photographs, however, are only of the views in which the microfossil rests.

Terminology

Most of the terms, used in the detailed description of the types in this paper, have, as in the previous papers, been borrowed from Wodehouse (1935), Erdtman (1943, 47, 52), and Faegri and Iverson (1950). But there

have been certain limitations to the use of this terminology. The difficulty of employing these terms accurately in the case of fossil pollen, which are loose is obvious. The proximal and distal sides of a living pollen can easily be made out as the grain can be observed in their tetrad arrangement. Similarly the disposition of the pores whether they are polar or equatorial in position can also be made out in living pollen. But these facts cannot be investigated satisfactorily with fossil pollen, which are loose and even distorted in shape. The other characters of the spores and pollen have lent themselves to be described in terms which have been adopted by Erdtman and others.

The question of Nomenclature, Classification and Comparison has already been discussed (Rao and Vimal, 1952, p. 596; Vimal, 1952, p. 136), and as in previous papers the spores and pollen, described in this paper have been placed mainly under Erdtman's classification (1947). It has not been possible to compare all the 36 types of spores and pollen described in this paper. But a few have been compared with living types with the help of published diagrams and descriptions. The comparisons can be summed up in the following table:—

Type Name	Family	Particular type to which compared
Monolites spm. 2	Polypodiaceæ	*Polypodium vulgare*
Monolites spm. 3	Polypodiaceæ	*Blechnum spicant*
Monolites spm. 4	Scizæaceæ	*Schizæa pennula*
Trilites spm. 1	Lygodiaceæ	*Lygodioisporites adriensis*
Verruconapites spm. 1	Potamogetonaceæ	*Potamogeton natans*
Monocolpites spm. 1–2	Palmæ	*Pritchardia minor*
Tricolpites spm. 2	Hamamelidaceæ	*Hamamelis virginiana*
Tricolpites spm. 3	Cornaceæ	*Cornus suecica*
Tricolporites spm. 1	Myrsinaceæ	*Myrsine Rockii*
Tricolporites spm. 2	Rosaceæ	
Tricolporites spm. 3	Rubiaceæ	*Kadua cordata*
Tetracolporites spm. 2	Myrsinaceæ	*Myrsine petiolata*
Pentacolporites spm. 1	Solanaceæ	*Nothocestrum peltatum*
Triorites spm. 2	Papilionaceæ	*Erythrina sandwicensis*
Triorites spm. 3	Ulmaceæ	*Celtis lævigata*
Triorites spm. 4	Betulaceæ	*Carpinus betulus*
Triorites spm. 5	Betulaceæ	*Ostrya virginiana*

Key to spores and pollen	Photo No.	Fig. No.	Page No.
1. Spores with Monolete scar (*Monolites*)			
Exospore reticulate, 29·7 μ×41·9 μ ..	1	1	182
Exospore smooth, 50·5 μ×29·7 μ	2	2–3	182
Exospore pitted, 51·1 μ×33·4 μ	3	4–5	183
2. Spore with Trilete scar (*Trilites*)			
Exospore smooth, 69·0 μ×77·2 μ	4	6	183
3. Pollen without any pore or furrow (*Nonaperturites*)			
Flattened, surface reticulate, 23·8 μ ..	5	7	183
Oblate spheroidal, surface reticulate, 17·9 μ ..	6	8	183
Oblate, surface verrucate, 41·6 μ	7	9	183
Spheroidal, surface echinate, 17·9 μ ..	8	10	185
Spheroidal, no definite features, 23·8 μ ..	9	11	185
GRAINS WITH FURROWS (*Colpites*)			
4. Pollen with one furrow (*Monocolpites*)			
Flattened, surface reticulate, 49·9 μ ..	10	12	185
Flat, surface smooth, 33·3 μ×20·2 μ ..	11	13	185
Flat, surface smooth, 44·1 μ×27·3 μ ..	12	14–15	185
5. Pollen with three furrows (*Tricolpites*)			
Spheroidal, surface reticulate, 19·1 μ ..	13–14	16–17	186
Subprolate, surface smooth, 23·8 μ×20·3 μ ..	15	18–19	186
Subprolate, surface smooth, 22·6 μ×13·6 μ ..	16–17	20–21	186
Prolate, surface smooth, 11·9 μ×15·6 μ ..	18	22–23	186
Surface reticulate, 44·1 μ×57·1 μ	19	24	187
6. Pollen with four furrows (*Tetracolpites*)			
Prolate, surface smooth, 23·8 μ×16·7 μ ..	20	25–26	187
7. Pollen with six furrows (*Hexacolpites*)			
Oblate, surface rugulate, 38·1 μ	21	27–29	187
8. Pollen with eight furrows (*Octacolpites*)			
Prolate spheroidal, surface smooth, 21·4 μ ..	22	30–31	187
GRAINS WITH PORATE FURROWS (*Colporites*)			
9. Pollen with three porate furrows (*Tricolporites*)			
Prolate spheroidal, surface smooth, 16·7 μ × 19·1 μ	23	32–33	187
Prolate spheroidal, surface smooth, 22·6 μ ..	24	34–35	188
Subprolate, surface Granular, 26·1 μ×22·1 μ	25	36–39	188
10. Pollen with four porate furrows (*Tetracolporites*)			
Prolate spheroidal, surface smooth, 23·8 μ ×21·4 μ	26	40–41	188
Subprolate, surface granular, reticulate, 23·8 μ	27	42–43	189

Key to spores and pollen	Photo No.	Fig. No.	Page No.
11. Pollen with five porate furrows (*Pentacolporites*)			
Prolate spheroidal surface smooth, 27·3 μ × 29·7 μ	28	44–46	189
Prolate, surface smooth, 23·8 μ × 21·4 μ ..	29	47–48	189
12. Pollen with six porate furrows (*Hexacolporites*)			
Oblate, surface rugulate, 40·4 μ	30	49–50	189
13. Pollen with seven porate furrows (*Septacolporites*)			
Oblate, surface rugulate, 38·1 μ	31	51–52	190
14. Pollen with eight porate furrows (*Octacolporites*)			
Suboblate, surface smooth, 23·8 μ	32–33	53–54	190
GRAINS WITH EQUATORIAL PORES (*Orites*)			
15. Pollen with three equatorial pores (*Triorites*)			
Oblate, surface reticulate, 35·7 μ	34	55–56	190
Spheroidal, surface granular, 35·7 μ ..	35	57–59	190
Oblate spheroidal, surface granular, 45·3 μ ..	36	60–61	191
Oblate spheroidal, surface smooth, 33·4 μ ..	37	62–63	191
GRAINS WITH NON-EQUATORIAL PORES (*Porites*)			
16. Pollen with three unequatorial pores (*Triporites*)			
Flaccid, surface smooth, 52·3 μ × 48·8 μ ..	38	64	191
17. Pollen with many pores (*Polyporites*)			
Flattened, surface scrobiculate, 49·4 μ ..	39–40	65	191

DESCRIPTION

Monolites (Erdtman, 1947)

Monolites spm. 2.—Spore large, heteropolar, brownish, bilateral. Lateral view (Pl. VIII, Fig. 1; Pl. IX, Fig. 1) plano-convex, 29·7 μ × 41·9 μ. Proximal view narrow. Exine intectate, 2·3 μ. Surface sculpture reticulate. These spores can be compared with the spores of *Polypodium vulgare*, (Erdtman, 1943, Fig. 483), in shape and surface sculpture. But in size they come nearer to *Polypodiaceæ* sp. *favous* R. Potonie (1934) which measures 42 μ × 28 μ (Potonie, 1951).

Monolites spm. 3.—Spore large, yellowish, heteropolar, bilateral. Lateral view (Pl. VIII, Fig. 2; Pl. IX, Fig. 2) concavo-convex, 50·5 μ × 29·7 μ. Proximal view (Pl. VIII, Fig. 3) narrow. Exine 1·5 μ thick, intectate. Surface sculpture smooth. The spore can be referred to the Polypodiaceæ. It resembles in form and surface sculpture the spores of *Blechnum* and falls well within the range of variations of size the spore of *B. spicant* 27–35 μ

To be substituted for Pages 197 & 198 in Vol. 38, No. 5, Section B, of the Proceedings

Key to spores and pollen	Photo No.	Fig. No.	Page No.
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Exospore reticulate, 29·7 μ × 41·9 μ ..	1	1	198
Exospore smooth, 50·5 μ × 29·7 μ	2	2–3	198
Exospore pitted, 51·1 μ × 33·4 μ	3	4·5	199
2. Spore with Trilete scar (*Trilites*)			
Exospore smooth, 69·0 μ × 77·2 μ	4	6	199
3. Pollen without any pore or furrow (*Nonaperturites*)			
Flattened, surface reticulate, 23·8 μ ..	5	7	199
Oblate spheroidal, surface reticulate, 17·9 μ ..	6	8	199
Oblate, surface verrucate, 41·6 μ	7	9	199
Spheroidal, surface echinate, 17·9 μ ..	8	10	200
Spheroidal, no definite features, 23·8 μ ..	9	11	200
GRAINS WITH FURROWS (*Colpites*)			
4. Pollen with one furrow (*Monocolpites*)			
Flattened, surface reticulate, 49·9 μ ..	10	12	200
Flat, surface smooth, 33·3 μ × 20·2 μ ..	11	13	200
Flat, surface smooth, 44·1 μ × 27·3 μ ..	12	14–15	200
5. Pollen with three furrows (*Tricolpites*)			
Spheroidal, surface reticulate, 19·1 μ ..	13–14	16–17	201
Subprolate, surface smooth, 23·8 μ × 20·3 μ ..	15	18–19	201
Subprolate, surface smooth, 22·6 μ × 13·6 μ ..	16–17	20–21	201
Prolate, surface smooth, 11·9 μ × 15·6 μ ..	18	22–23	201
Surface reticulate, 44·1 μ × 57·1 μ	19	24	201
6. Pollen with four furrows (*Tetracolpites*)			
Prolate, surface smooth, 23·8 μ × 16·7 μ ..	20	25–26	202
7. Pollen with six furrows (*Hexacolpites*)			
Oblate, surface rugulate, 38·1 μ	21	27–29	202
8. Pollen with eight furrows (*Octacolpites*) ..			
Prolate spheroidal, surface smooth, 21·4 μ ..	22	30–31	202
GRAINS WITH PORATE FURROWS (*Colporites*)			
9. Pollen with three porate furrows (*Tricolporites*)			
Prolate spheroidal, surface smooth, 16·7 μ × 19·1 μ	23	32–33	202
Prolate spheroidal, surface smooth, 22·6 μ ..	24	34–35	203
Subprolate, surface granular, 26·1 μ × 22·1 μ	25	36–39	203
10. Pollen with four porate furrows (*Tetracolporites*)			
Prolate spheroidal, surface smooth, 23·8 μ × 21·4 μ	26	40–41	203
Subprolate, surface granular, reticulate, 23·8 μ	27	42–43	204

Key to spores and pollen	Photo No.	Fig. No.	Page No.
11. Pollen with five porate furrows (*Penacolporites*)			
Prolate spheroidal surface smooth, 27·3 μ $\times$ 29·7 μ	28	44–46	204
Prolate, surface smooth, 23·8 $\mu \times$ 21·4 μ ..	29	47–48	204
12. Pollen with six porate furrows (*Hexacolporites*)			
Oblate, surface rugulate, 40·4 μ	30	49–50	204
13. Pollen with seven porate furrows (*Septacolporites*)			
Oblate, surface rugulate, 38·1 μ	31	51–12	205
14. Pollen with eight porate furrows (*Octacolporites*)			
Suboblate, surface smooth, 23·8 μ	32–33	53–54	205
GRAINS WITH EQUATORIAL PORES (*Orites*)			
15. Pollen with three equatorial pores (*Triorites*)			
Oblate, surface reticulate, 35·7 μ	34	55–56	205
Spheroidal, surface granular, 35·7 μ ..	35	57–59	205
Oblate spheroidal, surface granular, 45·3 μ ..	36	60–61	206
Oblate spheroidal, surface smooth, 33·4 μ ..	37	62–63	206
GRAINS WITH NON-EQUATORIAL PORES (*Porites*)			
16. Pollen with three unequatorial pores (*Triporites*)			
Flaccid, surface smooth, 52·3 $\mu \times$ 48·8 μ ..	38	64	206
17. Pollen with many pores (*Polyporites*) ..			
Flattened, surface scrobiculate, 49·4 μ ..	39–40	65	206

DESCRIPTION

Monolites (Erdtman, 1947)

Monolites spm. 2.—Spore large, heteropolar, brownish, bilateral. Lateral view (Pl. VIII, Fig. 1; Pl. IX, Fig. 1) plano-convex, 29·7 $\mu \times$ 41·9 μ. Proximal view narrow. Exine intectate, 2·3 μ. Surface sculpture reticulate. These spores can be compared with the spores of *Polypodium vulgare*, (Erdtman, 1943, Fig. 483), in shape and surface sculpture. But in size they come nearer to *Polypodiaceæ* sp. *favous* R. Potonie (1934) which measures 42 $\mu \times$ 28 μ (Potonie, 1951).

Monolites spm. 3.—Spore large, yellowish, heteropolar, bilateral. Lateral view (Pl. VIII, Fig. 2; Pl. IX, Fig. 2) concavo-convex, 50·5 $\mu \times$ 29·7 μ. Proximal view (Pl. VIII, Fig. 3) narrow. Exine 1·5 μ thick, intectate. Surface sculpture smooth. The spore can be referred to the Polypodiaceæ. It resembles in form and surface sculpture the spores of *Blechnum* and falls well within the range of variations of size the spore of *B. spicant* 27–53μ.

$\times 45$–$52\,\mu$ (Knox, 1951, p. 440, Fig. 12). Mrs. Chitaley has described a similar but smaller spore from the Intertrappean cherts of Mohgaon Kalan in Madhya Pradesh, as *Monolites* spm. Wilson and Webster have also described some monolete spores from Montana in U.S.A. under the name of *Lævigatosporites ovatus* sp. nov. and *Lævigatosporites gracilis* sp. nov., and from three N. Central Wisconsin bogs under the name of *Thelypteris* sp. All of them are smaller than mine. Pratap Singh (1952, Text-Fig. 12) has described spores of similar but smaller form ($30\,\mu \times 18\,\mu$) from the Salt Range (Pakistan).

Monolites spm. 4.—Spore large, yellowish, heteropolar, bilateral. Lateral view (Pl. VIII, Fig. 4; Pl. IX, Fig. 3) plano-convex, $51{\cdot}1\,\mu \times 33{\cdot}4\,\mu$. Proximal view (Pl. VIII, Fig. 5) narrow. Exine $2{\cdot}4\,\mu$ thick, intectate. Surface sculpture scrobiculate. The spores can be referred to the Schizæaceæ in form surface sculpture, and approximately in size to those of *Schizæa pennula* (Selling, 1944, Pl. III, Figs. 28–31). Fossil spores similar in form and surface sculpture, but much bigger in size ($78\,\mu \times 114\,\mu$) have been described by Selling (1946, Figs. 40–41) from Hawaii.

Trilites (Erdtman, 1947)

Trilites spm. 1.—Spore large, tetrahedral, brown, heteropolar, radiosymmetric. Proximal view (Pl. VIII, Fig. 6; Pl. IX, Fig. 4) subtriangular $69{\cdot}0\,\mu \times 77{\cdot}2\,\mu$. Triradiate mark very clear and reach a little away from the margin. Exospore $2{\cdot}5\,\mu$ thick smooth. No perine or perisporium is visible. Spores similar in shape and approximately of the same size ($63\,\mu - 75\,\mu$) have been described by Potonie and Gell (1932) under the name of *Lygodioisporites adriennis* (Potonie, 1951).

Nonaperturites (Erdtman, 1947)

Reticulonapites spm. 2.—Grain small, brownish, yellow, round, flattened, apolar, radiosymmetric (Pl. VIII, Fig. 7; Pl. IX, Fig. 5) $23{\cdot}8\,\mu$ across. Exine $1{\cdot}2\,\mu$ thick. Surface sculpture reticulate.

Reticulonopites spm. 3.—Grain small, brown, oblate spheriodal, apolar, radiosymmetric (Pl. VIII, Fig. 8; Pl. IX, Fig. 6) $17{\cdot}9\,\mu$ across. Surface sculpture reticulate.

Verruconapites spm. 1.—Grain large, yellowish, oblate, apolar, radiosymmetric (Pl. VIII, Fig. 9; Pl. IX, Fig. 7) $41{\cdot}6\,\mu$ across. Exine thick, intectate. Surface sculpture verrucate. The grain can be compared with the pollen of *Potamogeton natans*. Similar grains have also been found in the Palana (Rao and Vimal, 1950) and Dandot (Salt Range) lignites (Vimal, 1952).

Nonaperturites spm. 1.—Grain small, brown, spheroidal, apolar, radiosymmetric. This interesting grain (Pl. VIII, Fig. 10; Pl. IX, Fig. 8) has a number of spine-like processes 5·7μ long and about a micron broad covering the entire surface, which is 17·9 μ in diameter. These are free from each other and arise from the exine which is about a micron thin. The affinites of the grain are not clear, but it is tentatively classed as a pollen grain. A triradiate mark or a definite furrow or pore are not also clear. So it is placed under *Nonaperturites*.

Nonaperturites spm. 2.—Grain small, brown, spheroidal, apolar, radiosymmetric, (Pl. VIII, Fig. 11; Pl. IX, Fig. 9), 23 ·8 μ across. Exine 2·3 μ thick. Surface shows thin lines making up a reticulate pattern with large meshes. This pattern is not shown in the photograph but is seen in the figure. In fact this feature may be due to defective preservation and may not be a natural feature of the grain.

Monocolpites (Erdtman, 1947)

Monocolpites spm. 1.—Grain large, yellowish, circular, flattened, apolar, bilateral, (Pl. VIII, Fig. 12; Pl. IX, Fig. 10), 49·9 μ across. Ectœxine 3·5 μ; endœxine 1·2 μ thick. Surface sculpture tectate, reticulate. A long narrow furrow (Pl. VIII, Fig. 12) is seen almost in the middle of the grain.

Monocolpites spm. 2.—Grain small, yellow, flat, apolar, bilateral (Pl. VIII, Fig. 13; Pl. IX, Fig. 11) 33·3 μ long and 20·2 μ broad; slightly broader at one end. Furrow 27·3 μ long and 3·5 μ broad. Since the side bearing the furrow can not be definitely established to be the distal side, the grain is not placed under the *Monosulcites*. Ectœxine about a micron thin; light yellow; endœxine 1·2 μ thick, darker. Surface sculpture smooth. The grain resembles in appearance the monosulcate grain of Palms like *Borassus flabelliformis* which grows wild in India or *Pritchardia minor* (Becc.) (Selling, 1947, p. 335, Figs. 865–68) absent in Indian flora. *Monocolpites* spm. 2 is however comparatively more flattened than the pollen of the other two. Besides this it has not been possible to make out definitely the agreement in size of the grain.

Monocolpites spm. 3.—Grain large, flat, yellow, peroblate, apolar, bilateral (Pl. VIII, Fig. 14; Pl. IX, Fig. 12) 44·1 μ × 27·3 μ. A long narrow colpæ is seen along the longer axis of the grain on one side only. Side view narrow (Pl. VIII, Fig. 15). Ectœxine thin, light yellow; endœxine 1·2μ thick, darker. Surface sculpture smooth. The grain is very similar to *Monocolpites* spm. 2, but differs from it in having a narrower furrow and offers a closer comparison with *Pritchardia minor* (Becc.) in shape, size and surface features. Similar

Monocolpites types of grain from Intertrappean cherts of Mohagaon Kalan have been referred by Mrs. Chitaley (1951) to the Palmæ.

Tricolpites (Erdtman, 1947)

Tricolpites spm. 2.—Grain small, spheroidal, brownish, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 16; Pl. IX, Fig. 13) round, 19·1μ across, three lobed with rounded poles. Equatorial view (Pl. VIII, Fig. 17; Pl. IX, Fig. 14) round with one flange in focus. Furrows short and narrow. Exine 2·3μ thick. Surface sculpture reticulate. The grain can be compared with the pollen of *Hamamelis virginiana* (Erdtman, 1943, p. 102, Figs. 216–17) in all respects except furrows are shorter and narrower in my grain.

Tricolpites spm. 3.—Grain small, brown, subprolate, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 18) round, three lobed, 17·9μ across. Equatorial view (Pl. VIII, Fig. 19; Pl. IX, Fig. 15) oblong, 23·8$\mu\times$ 20·3μ with one flange in focus. Flanges convex on the outer surface. Furrows long and very thin. The furrow is interrupted by pore like areas (Pl. VIII, Fig. 19), but it is not definitely clear if these are really pores. Grains like these but larger in size has also been recovered from Palana lignites (Rao and Vimal, 1950). Such types of grain have also been described from the Salt Range oil shales (P. Singh, 1952, p. 419, Text-Fig. 14). The grain can be compared in surface sculpture and approximately in size also (29$\mu\times$19μ) with the pollen of *Cornus suecica* (Erdtman, 1943, p. 87, Fig. 144). In its equatorial view my grain resembles in form and size also the grain described by Potonie (1931) as *Pollenites megæxactus* (Potonie, 1951).

Tricolpites spm. 4.—Grain small, pale yellow, subprolate, isopolar, radiosymmetric. Equatorial view (Pl. VIII, Fig. 20; Pl. IX, Fig. 16) oblong with one flange in focus, 22·6$\mu\times$13·6μ. Furrow long and narrow (*f*, Pl. IX, Fig. 17). Each flange (*fl*, Pl. IX, Fig. 16) is 8·3μ broad and slightly convex on its outer surface. Polar view (Pl. VIII, Fig. 21) round, three lobed 15·7μ across. Exine 1·2μ thick. Surface smooth.

Tricolpites spm. 5.—Grain small, yellowish, prolate, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 22) rounded, three lobed 13·1μ across. Equatorial view (Pl. VIII, Fig. 23; Pl. IX, Fig. 18) oblong 11·9$\mu\times$15·6μ with one flange in focus. Flange slightly convex on its outer surface, spindle shaped, 5·7μ broad in the middle. Ectœxine and endœxine each about a micron thin. Surface smooth.

Tricolpites spm. 6.—Grain large, brownish, isopolar, radiosymmetric. Oblique polar view (Pl. VIII, Fig. 24; Pl. IX, Fig. 19) 44·1$\mu\times$57·1μ, three lobed. Furrows very broad, long reaching the poles. Exine thick. Surface

sculpture reticulate. There is some resemblence in size and shape between this grain and the *Type* 9 described from Palana (Rao and Vimal, 1950, p. 84).

Tetracolpites (Erdtman, 1947)

Tetracolpites spm. 1.—Grain small, brown, prolate, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 25) squarish, four lobed. Equatorial view with two furrows in focus (Pl. VIII, Fig. 26; Pl. IX, Fig. 20), $23{\cdot}8\,\mu \times 16{\cdot}7\,\mu$ oblong. Furrows $17{\cdot}9\,\mu$ long, narrow and terminate at quite a distance from the poles. Exine $1{\cdot}2\,\mu$ thick. Surface smooth.

Hexacolpites (Erdtman, 1947)

Hexacolpites spm. 1.—Grain large, brown, oblate, isopolar, radiosymmetric. Pl. VIII, Fig. 27; Pl. IX, Fig. 21 show the round polar view, $38{\cdot}1\,\mu$ across, with only four furrows (*f*), and four flanges, slightly convex on the outer side. Equatorial view (Pl. VIII, Fig. 28) elliptic, $38{\cdot}1\mu \times 26{\cdot}1\mu$. Furrows (*f*) short and narrow. The thickness of the wall of each flange is seen in the photo, and this can be seen to be continuous with the rugulate pattern of the surface of the exine (Pl. VIII, Fig. 29 showing oblique polar view). Surface sculpture rugulate.

Octacolpites (Erdtman, 1947)

Octacolpites spm. 2.—Grain small, yellow, prolate spheroidal, isopolar, radiosymmetric. Equatorial view (Pl. VIII, Fig. 30) oblong, $21{\cdot}4\,\mu \times 22{\cdot}6\,\mu$ with rounded poles and only four furrows in focus. The furrows are clearly seen in Pl. IX, Fig. 22, which shows an oblique polar view of the grain. Furrows long and very thin and not reaching the poles. Polar view (Pl. VIII, Fig. 31), $17{\cdot}2\,\mu$ across, eight lobed. Exine $1{\cdot}2\,\mu$ thick. Surface smooth.

Tricolporites (Erdtman, 1947)

Tricolporites spm. 1.—Grain small, brown, prolate spheroidal, isopolar, radiosymmetric. Pl. VIII, Fig. 32; Pl. IX, Fig. 23 show the equatorial view of the grain with two pores (*p*) in focus. One of them is situated on the furrow (*f*) and is seen in surface view, the other is seen in lateral view. The grain measures $16{\cdot}7\mu \times 19{\cdot}1\mu$ in this aspect. Polar view (Pl. VIII, Fig. 33) round $16{\cdot}7\,\mu$ across, poles rounded; furrows long, narrow and reaching the poles. Pores large (grandi-porate), rounded, $2{\cdot}3\,\mu$ in diameter. Exine $1{\cdot}2\,\mu$ thick. Surface smooth. Grains exactly similar in structure but of varying sizes ($23{\cdot}8\,\mu \times 28{\cdot}5\,\mu$; $21{\cdot}5\,\mu \times 29{\cdot}7\,\mu$; $24{\cdot}9\,\mu \times 32{\cdot}1\,\mu$) have also been found, they have been referred to the same sporomorphic type as it is likely that they represent variations of the same species of pollen. This kind of variation is found in pollen of living species. The grain resembles in size, shape

and surface sculpture the pollen of *Myrsine Rockii* (Selling, 1947, p. 251, Pl. 32, Figs. 512–13), but *M. Rockii* is generally supposed to be tetracolporate and occasionally tricolporate. It is not unlikely that my grain represent such unusual grains of *M. Rockii* particularly in view of the fact that the tetracolporate grains of the same characters are also found in one of the same slide (*Tetracolporites* spm. 2). *Tricolporites* spm. 1 has also been found in some samples of Palana lignites (Vimal, Mss.).

Tricolporites spm. 2.—Grain small, yellow, prolate spheroidal, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 34; Pl. IX, Fig. 24) roundish, three lobed, 22·6μ across. Equatorial view (Pl. VIII, Fig. 35) oblong, showing one of the pores in focus. Furrows long (longi-colpate), narrow nearly reaching the poles. Pores almost round 5·9$\mu \times$4·7μ big (grandi-porate) appear to be covered by a thin membrane. Exine 1·5μ thick. Surface smooth. There is a resemblance in size, shape, surface and furrow characters between this grain and *Tricolporites* (*Rosacidites*) spm. from the Deccan Intertrappean cherts described by Mrs. Chitaley (1951, Pl. XIV, Fig. 19).

Tricolporites spm. 3.—Grain small, yellowish, subprolate, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 36) round, three lobed, 23·8μ across. Pl. VIII, Fig. 37; Pl. IX, Fig. 25 show the equatorial view. The grain looks oblong, measures 26·1$\mu \times$22·1μ and two elliptic pores can be made out. Poles round, flanges slightly convex on their outer surface. Pl. VIII, Fig. 38 shows the grain with one pore and furrow in view. The pores in this grain are, however, not very clear. Furrows long and narrow. Ectœxine and endœxine each 1·2μ thick. Surface sculpture granular. The grain can be compared with the pollen of Rubiaceæ, and agrees with that of *Kadua cordata* (cham. et. Schlecht) (Selling, 1947, p. 300, Pl. 43, Figs. 686–88). Mrs. Chitaley (1951, Pl. XIV, Fig. 20; Text-Fig. 21) has figured from the Deccan Intertrappean cherts pollen grains similar to mine in size and shape. Grains exactly like those of *Tricolporites* spm. 3, but having four fourrows have also been found (Pl. VIII, Fig. 39). I think these are but variants of the same type, so they have not been described separately. *Tricolporites* spm. 3 has also been found in the Palana lignites (Vimal, Mss.).

Tetracolporites (Erdtman, 1947)

Tetracolporites spm. 2.—Grain small, yellow, prolate, spheroidal, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 40) roughly squarish, 23·8μ $\times$21·4μ, colpæ at the angles. Equatorial view (Pl. VIII, Fig. 41; Pl. IX, Fig. 26) oval, 23·8$\mu \times$27·3μ with one furrow (*f*) and two flanges (*fl*) in focus. Furrows long (longi. colpate) and narrow. Pores (*p*) big (grandi-porate),

round, 2·6 μ in diameter. Exine 2·4 μ thick. Surface smooth. The grain resembles the pollen of *Myrsine petiolata* Hosaka in size, shape and surface sculpture (Selling, 1947, p. 25).

Tetracolporites spm. 3.—Grain small, yellowish, subprolate, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 42) more or less squarish, 23·8 μ across, with four colpæ diagonally placed. Poles slightly rounded or obtuse. Equatorial view (Pl. VIII, Fig. 43; Pl. IX, Fig. 27) oblong, 28·5 $\mu \times$ 22·6 μ with one porate furrow in focus. The other two pores are also seen in the photo. Exine 2·3 μ thick. Surface sculpture faintly granular reticulate.

Pentacolporites (Erdtman, 1947)

Pentacolporites spm. 1.—Grain small, yellowish brown, prolate spheroidal, isopolar, radiosymmetric. Equatorial view (Pl. VIII, Fig. 44; Pl. IX, Fig. 28) oblong, 27·3 $\mu \times$ 29·7 μ three of the five furrows are in focus. The other two porate furrows of the same grain are also shown in Pl. VIII, Fig. 45. Polar view (Pl. VIII, Fig. 46) rounded, five angled, 27·3 μ across. Pores round 4·7 μ in diameter and seems to be covered over by a layer of the exine (*see* Pl. VIII, Fig. 44). This explains why the pore is not very distinct. Furrows long and thin. Exine 2·3 μ thick. Surface smooth. The grain can be compared with the pollen of *Nothocestrum peltatum* (Selling, 1947, p. 282, Pl. 39, Figs. 606–08).

Pentacolporites spm. 2.—Grain small, brown, prolate, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 47) round, five lobed, 23·8 $\mu \times$ 21·4 μ, lobes convex on the outer surface. Equatorial view (Pl. VIII, Fig. 48; Pl. IX, Fig. 29) oblong, 32·1 $\mu \times$ 23·8 μ with three porate furrows in focus. Furrows long (longi-colpate) almost reaching the rounded poles, pores broad (lati-porate) 3·5 $\mu \times$ 5·9 μ. Exine 2·3 μ thick. Surface smooth. Similar looking pollen grains have been described by Potonie (1951, Pl. VIII, Fig. 48), but further comparison is difficult as I have not been able to see the paper describing these grains.

Hexacolporites (Erdtman, 1947)

Hexacolporites spm. 3.—Grain large, brown, oblate, isopolar, radiosymmetric. Equatorial view (Pl. VIII, Fig. 49) broadly elliptic, 40·4 $\mu \times$ 23·8 μ showing two furrows only, one fully and the other partly. Furrows broad (lati-colpate) and short (brevi-colpate); pores large (grandi-porate). Polar view (Pl. VIII, Fig. 50; Pl. IX, Fig. 30) round, 40·4 μ shows the six lobes, subdivided into two or three lobes. Ectœxine thin, smooth, lighter in colour; endœxine thick and darker. Surface sculpture rugulate.

Septacolporites (Erdtman, 1947)

Septacolporites spm. 1.—Grain large, brown, oblate, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 51; Pl. IX, Fig. 31) round, seven lobed, 38·1μ across. Equatorial view (Pl. VIII, Fig. 52) transversely elliptic. Furrows seven, broad and short, enclose a pore each. Pores broad and large. Exine thick. Surface sculpture rugulate.

Octacolporites (Erdtman, 1947)

Octacolporites spm. 1.—Grain small, yellowish, suboblate, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 53; Pl. IX, Fig. 32) round, lobed, 23·8μ across. Equatorial view (Pl. VIII, Fig. 54; Pl. VIII, Fig. 33) broadly elliptic, biconvex 17·9μ along the polar axis and 23·8μ along the equator of the grain, which is ridged. Four furrows and the intervening flanges are seen in Pl. VIII, Fig. 54. Furrows long and narrow. Along the ridge is a thin slit going all round, this may be a transverse furrow or the transversely elongated pores touching each other. Ecto and endœxine each 1·2μ thick, but the former being lighter in colour. Surface smooth.

Triorites (Erdtman, 1947)

Triorites spm. 2.—Grain small, yellow, oblate, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 55; Pl. IX, Fig. 34) triangular, 35·7μ across, with three pores, one at each angle, sides straight or slightly convex. Equatorial view (Pl. VIII, Fig. 36) ellipsoidal, 26·1$\mu \times$ 39·3μ, shows one pore in focus. Pores large, roundish, 4·7$\mu \times$ 5·9μ. Ectœxine and endœxine each 1·2μ thick. Surface sculpture reticulate. The grain can be compared with the pollen of *Erythrina sandwicensis* Degener (Selling, 1947, p. 150, Pl. 10, Figs. 190–92) in shape, size and surface sculpture and the coarse reticulation seen in the polar area.

Triorites spm. 3.—Grain small, yellowish brown, spheroidal, apolar, radiosymmetric. Pl. VIII, Fig. 57 shows the grain drawn at a higher focus with only one pore in focus. The grain measures 33·3$\mu \times$ 35·7μ in this aspect. Pl. VIII, Fig. 58 and Pl. IX, Fig. 35 show the same grain at a lower focus with the other two pores in view. Pores large, aspidate, but not protruding above the surface, roundish, 5·9$\mu \times$ 4·5μ. Exine 1·2μ thick, lighter in colour. Subexineous thickening (*s.e.*, Pl. VIII, Fig. 59) present, but darker and thicker (3·5μ) near the pores. Surface covered with small, closely packed grains, giving a distinct granular texture to the exine. The grain can be compared with the pollen of *Celtis lævigata* K. koch. (*Celtis mississipiensis*, Spach.) which is of the same size and also possess aspidate pores (Wodehouse, 1935, p. 389).

Triorites spm. 4.—Grain large, yellow, flaccid, roughly oblate spheroidal, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 60; Pl. IX, Fig. 36) rounded, $45 \cdot 3\mu \times 40 \cdot 4\mu$. Equatorial view (Pl. VIII, Fig. 61) roundish, $38 \cdot 1\mu \times 45 \cdot 3\mu$ with only one pore in focus. Pores equatorial, elongated, $4 \cdot 7\mu \times 11 \cdot 9\mu$. Exine $2 \cdot 3\mu$ thick. Surface sculpture granular. The grain can be compared with the pollen of *Carpinus betulus* (Erdtman, 1943, p. 76, Figs. 76–78). Grains similar to *Triorites* spm. 4 have also been found in the lignites of Palana (Rao and Vimal, 1952) and Dandot (Vimal, 1952).

Triorites spm. 5.—Grain small, brown oblate spheroidal, isopolar, radiosymmetric. Polar view (Pl. VIII, Fig. 62; Pl. IX, Fig. 37), $33 \cdot 3\mu$ across, subtriangular, with the pores at the angles. Pores large, round, aspidate (annulate), $2 \cdot 4\mu$ in diameter, slightly raised above the surface. Arci clearly seen. In Pl. VIII, Fig. 63 which represents the equatorial view, two pores are seen on the two sides, while the third one is seen in surface view. Exine $1 \cdot 2\mu$ thick. Subexineous thickening (*s.e.* Pl. VIII, Fig. 62) $2 \cdot 4\mu$ thick. Surface smooth. The grain resembles the pollen of *Ostrya virginiana* (Erdtman, 1943, p. 79, Figs. 91–93) in all respects. Such grains have also been discovered from the Eocene beds of Erie basin (Sears, 1930, Figs. 25–25 A).

Triporites (Erdtman, 1947)

Triporites spm. 2.—Grain large, brownish yellow, spherical, flaccid, apolar, radiosymmetric. Pl. VIII, Fig. 64; Pl. IX, Fig. 38 show the grain ($52 \cdot 3\mu \times 48 \cdot 8\mu$) with two pores in focus. Pores round, large, aspidate, $7 \cdot 1\mu$ in diameter. Ectœxine and endœxine each $1 \cdot 2\mu$ thick. Surface smooth. This grain differs from *Triporites* spm. 1 (Rao and Vimal, *Curr. Sci.*, 1952, p. 302, Figs. 5–6) in being bigger in size and having smooth surface and aspidate pores.

Polyporites (Erdtman, 1947)

Polyporites spm. 1.—Grain large, yellow, spherical, flattened, apolar, radiosymmetric, Pl. VIII, Fig. 65; Pl. IX, Fig. 39, $49 \cdot 9\mu$ across. Pores roundish, more than the fifteen, distributed all over the surface (Pl. IX, Fig. 40). Exine $3 \cdot 5\mu$ thick. Surface sculpture scrobiculate.

DISCUSSION AND CONCLUSION

The Warkalli lignites are according to Krishnan (1949, p. 464) Miocene so younger than the Eocene lignites of Palana and Dandot. A comparison of the various types found in these three lignites, shows that the *Nonaperturites* (*Potamogeton*); *Triorites* (*Betula*, *Carpinus*) and the *Hexacolpites* types of the pollen are common to all the above three lignites. The *Trilites* type found abundantly in Dandot are represented by one type in Warkalli

and are totally absent in Palana. The *Triorites* spm. *Type No.* 16, of pollen found in Palana (Rao and Vimal, 1952) are totally absent in Warkalli as well as in Dandot lignites. The *Colporites* types are found both in Palana and Warkalli, but are absent in Dandot. *Monolites* type have been found only in Warkalli lignites. Winged pollen are conspicuous by their absence in all the above three lignites.

Some of the types (*Monolites* spm. 2, *M.* spm. 3, *M.* spm. 4; *Trilites* spm. 1; *Verruconapites* spm. 1; *Monocolpites* spm. 2 and spm. 3; *Tricolpites* spm. 3, *Tricolpites* spm. 6; *Tricolporites* spm. 1, spm. 2 and spm. 3; *Triorites* spm. 3, spm. 4, and spm. 5) described in the Warkalli lignites have also been found in other Tertiary deposits of India (Palana, Intertrappean cherts of Mohgaon Kalan, Madhya Pradesh); and other countries (Dandot lignites, and oil shales from Saline series in Pakistan; Oil shales of Green River, Eocene beds of Erie Basin and Fort Union coal of Montana in U.S.A.; Brown coals of Europe).

A rather significant fact is that most of the types, so far observed in the Warkalli lignites have a smooth surface—a feature regarded by Wodehouse (1935) and Faegri and Iverson (1951) as characteristic of anemophilous pollen. This feature at once makes one suspect that many of the pollen found in these lignites perhaps did not belong to an *in situ* flora but may have been blown into it by air currents. In view of this it would be unwise to speculate about the climate or the flora of the area in particular. Still the collection of pollen grains indicate in a general way the possible composition of the Miocene flora of this part of India.

In the absence of clearly recognisable tree pollen in this material, against which other pollen could be counted, the usual method of pollen analysis could not be done. Yet to find out the relative frequency of the different types, five different slides picked at random and from different macerations were examined and their pollen grains counted and classified, as in the following table. Attention may be drawn to the fact that the *Tetracolporites*, the *Tricolporites*, the *Septacolpites*, *Octacolpites* and the *Triorites* spm. 1 type of pollen seem to occur in abundance. Beyond this I do not propose to draw any further inferences from this table, as there are not enough reliable data.

Slide No.		1	2	3	4	5
Total No. of grains counted	..	100	50	90	80	45
Monolites spm. 1	..	2	2	..	..	..
Monolites spm. 4	..	1	..	..	..	1

Slide No.		1	2	3	4	5
Triorites spm. 1	..	11	3	12	14	3
Monocolpites spm. 2–3	..	2	2	4	2	2
Tricolpites spm. 2	..	2	3	1	..	2
Septacolpites spm. 1	..	11	4	7	8	4
Octacolpites spm. 1	..	10	3	7	9	3
Tricolporites spm.	..	15	7	10	6	4
Tetracolporites spm.	..	20	12	28	21	7
Hexacolporites spm. 2	..	1	..	1	1	1
Trilites spm. 1	..	1	..	..	2	..
"Unknowns"	..	24	14	20	17	18

SUMMARY

In the present paper about thirty-six types of different spores and pollen have been described, which were recovered from some samples of lignites from Warkalli in Travancore, India. Out of these three are *Monolites* type of spores and one *Trilites* type. Rest are all pollen grains classed under *Nonaperturites*, *Monocolpites*, *Tricolpites*, *Tetracolpites*, *Hexacolpites*, *Septacolpites*, *Octacolpites*, *Tricolporites*, *Tetracolporites*, *Pentacolporites*, *Hexacolporites*, *Septacolporites*, *Octacolporites*, *Triorites*, *Triporites* and *Polyporites*.

Some of the spores have been compared with *Polypodium vulgare*, *Blechnum spicant* and *Schizæa pennula*, while some of the pollen grains have been compared with the following genera of living plants: *Potamogeton*, *Borassus*, *Hamemelis*, *Myrsine*, *Cornus*, *Kadua*, *Nothocestrum*, *Erythrina*, *Celtis*, *Carpinus* and *Ostrya*.

Some of the types described in this paper have also been reported from other Tertiary deposits of India (Palana lignites, Intertrappean cherts of Mohgaon Kalan) and other countries (Dandot lignites and Oil shales of Saline series in Pakistan; Eocene beds of Erie Basin, Oil shales of Green River, and Fort Union coal of Montana in U.S.A., Brown coals of Europe).

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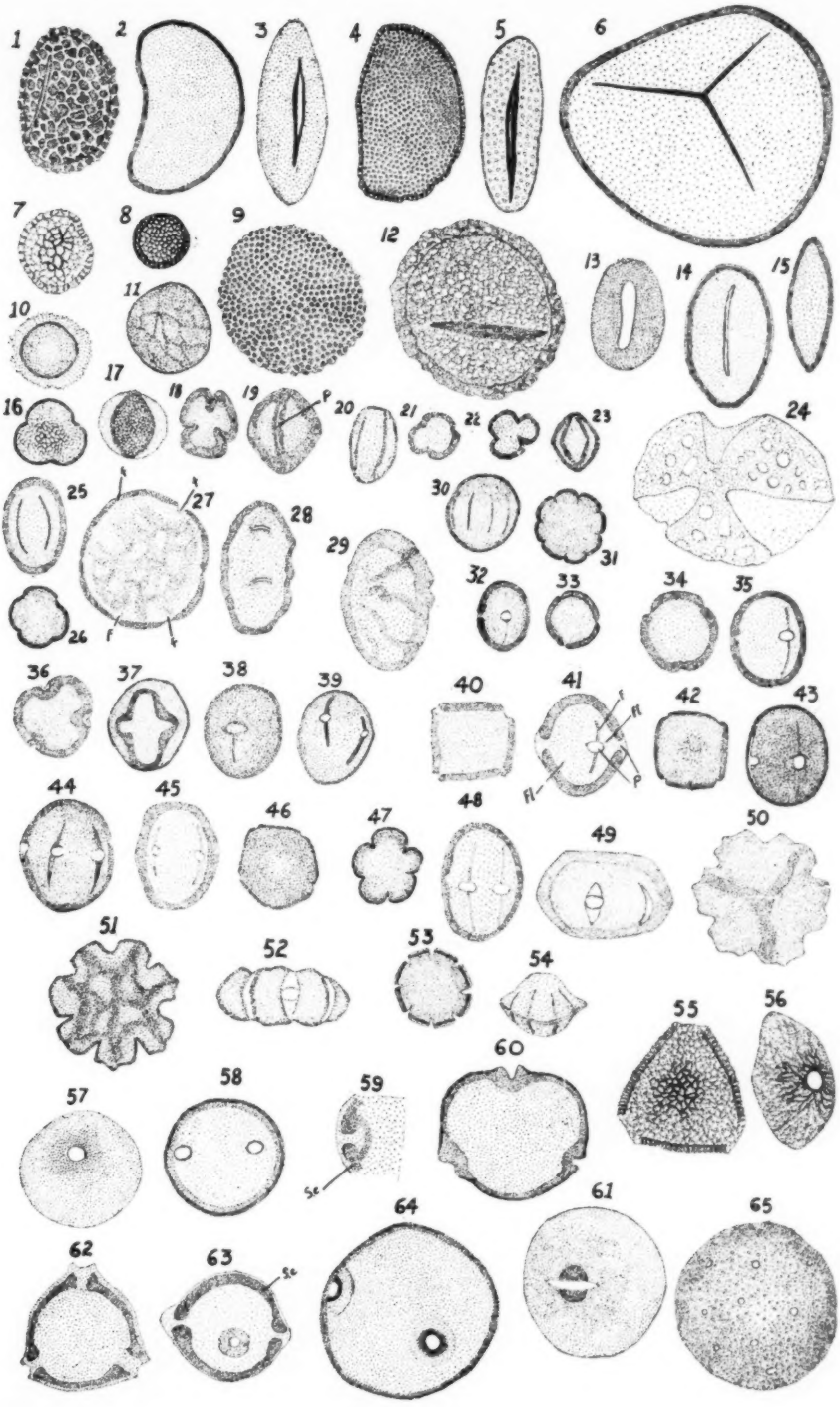
EXPLANATION OF PLATES

PLATE VIII

Monolites 1–5; *Trilites* 6; *Nonaperturites* 7–11; *Monocolpites* 12–15; *Tricolpites* 16–24; *Tetracolpites* 25–26; *Hexacolpites* 27–29; *Octacolpites* 30–31; *Tricolporites* 32–39; *Tetracolporites* 40–43; *Pentacolporites* 44–48. *Hexacolporites* 49–50; *Septacolporites* 51–52; *Octacolporites* 53–54; *Triorites* 55–63; *Triporites* 64; *Polyporites* 65. All figures are magnified 650 times.

PLATE IX

FIGS. 1–40. Fig. 1. *Monolites* spm. 2, Lateral view. Fig. 2. *Monolites* spm. 3, Lateral view. Fig. 3. *Monolites* spm. 4, Lateral view. Fig. 4. *Trilites* spm. 1, Proximal view. Fig. 5. *Reticulonapites* spm. 2. Fig. 6. *Reticulonapites* spm. 3. Fig. 7. *Verruconapites* spm. 1. Fig. 8. *Nonaperturites* spm. 1. Fig. 9. *Nanaperturites* spm. 2. Fig. 10. *Monocolpites* spm. 1. Fig. 11. *Monocolpites* spm. 2. Fig. 12. *Monocolpites* spm. 3. Fig. 13. *Tricolpites* spm. 2, Polar view. Fig. 14. *Tricolpites* spm. 4, Equatorial view. Fig. 15. *Tricolpites* spm. 3, Equatorial view. Fig. 16. *Tricolpites* spm. 4, Equatorial view, *fl*, flange. Fig. 17. *Tricolpites* spm. 4, Equatorial view, *f*, furrow. Fig. 18. *Tricolpites* spm. 5, Equatorial view. Fig. 19. *Tricolpites* spm. 6, Oblique polar view. Fig 20. *Tetracolpites* spm. 1, Equatorial view. Fig. 21. *Hexacolpites* spm. 1, Polar view, *f*, furrow. Fig. 22. *Octacolpites* spm. 2, Oblique polar view. Fig. 23. *Tricolporites* spm. 1, Equatorial view, *f*, furrow; *p*. pore. Fig. 24. *Tricolporites* spm. 2, Polar view. Fig. 25. *Tricolporites* spm. 3, Equatorial view. Fig. 26. *Tetracolporites* spm. 2, Equatorial view, *p*. pore; *fl*. flange; *f*, furrow. Fig. 27. *Tetracolporites* spm. 3. Equatorial view. Fig. 28. *Pentacolporites* spm. 1, Equatorial view. Fig. 29. *Pentacolporites*, 2, Equatorial view. Fig. 30. *Hexacolporites* spm. 3, Polar view. Fig. 31. *Septacolporites* spm. 1, Polar view. Fig. 32. *Octacolporites* spm. 1, Polar view. Fig. 33. *Octacolporites* spm. 1, Equatorial view. Fig. 34. *Triorites* spm. 2, Polar view. Fig. 35. *Triorites* spm. 3. Fig. 36. *Triorites* spm. 4, Polar view, Fig. 37. *Triorites* spm. 5, Polar view. Fig. 38. *Triporites* spm. 2. Fig. 39. *Polyporites* spm. 1. Fig. 40. *Polyporites* spm. 1. All photographs are from untouched negatives and unless otherwise stated are magnified 680 times.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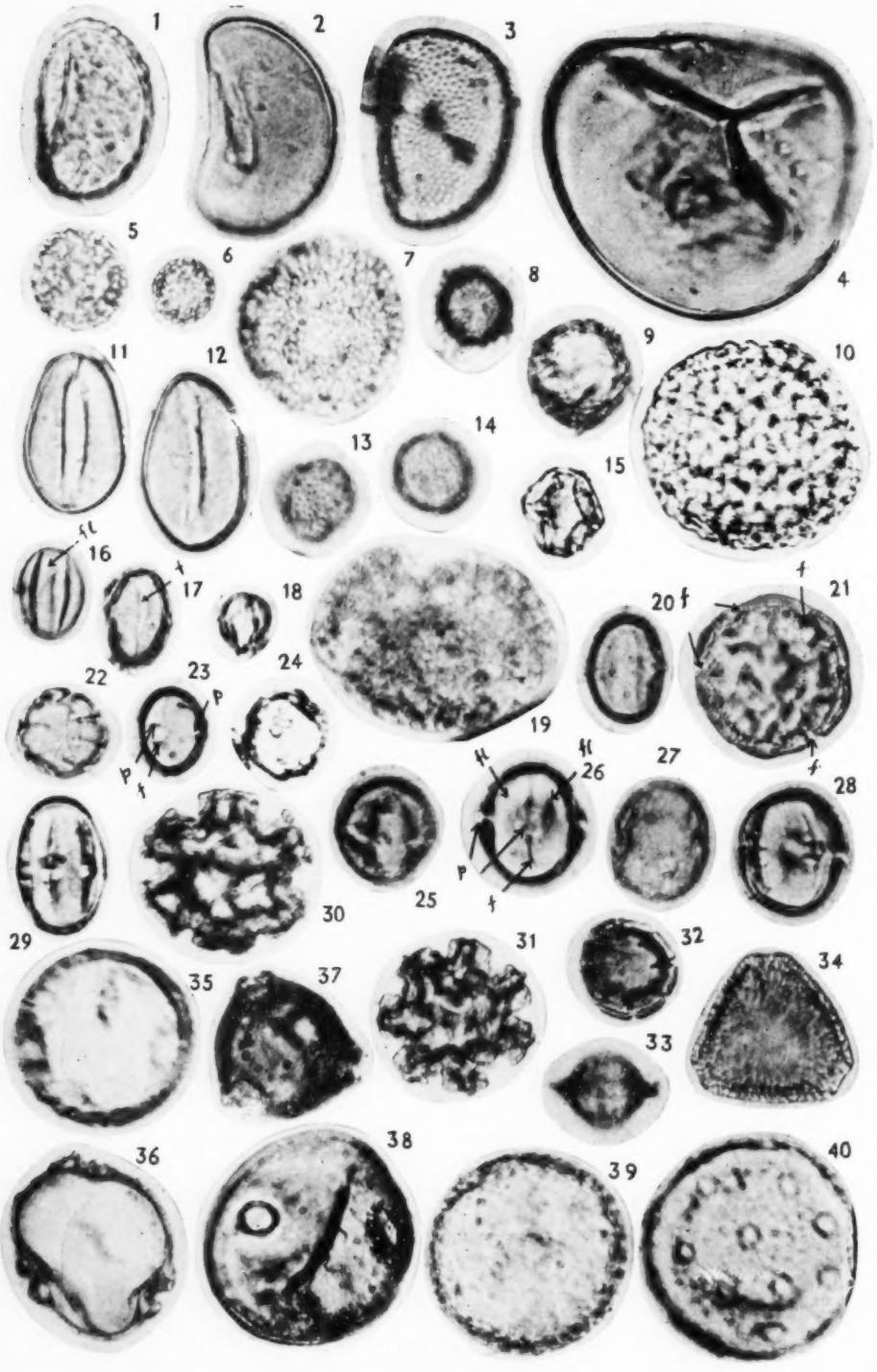
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PRELIMINARY OBSERVATIONS ON THE CONTROL OF AQUATIC INSECTS IN NURSERY PONDS*

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(Communicated by Dr. H. Srinivasa Rao, F.A.SC.)

THE majority of the aquatic insects are known to prey upon and destroy large numbers of young carp fry in nursery ponds.[1, 3] The Notonectids, *Anisops* spp. (*A. barbata* Brooks, *A. Kempi* Brooks) generally occur in thousands in carp nurseries in Orissa and Bengal during the monsoon season and do immense harm to the young carp fry which are then transplanted in these ponds for culture. These insects are almost of the same size as the week-old carp fry and are, therefore, difficult to segregate from the latter by sieving or other similar methods. When carp fry are netted from nursery ponds these insects are also caught in appreciable numbers and when kept together in the limited space of the *hapa* or *hundi*, they become extremely destructive to the fry. Though aquatic in habitat these Notonectids can fly out of water and go to neighbouring ponds as well. They very often come up to the surface of water for taking air for breathing. Spraying oil on water surface to control insects which come up to take atmospheric air, is a well-known principle, and a routine practice in malaria control. It is also reported that some fish farmers in Bengal use mustard oil to segregate Notonectids and carp fry when they are kept in *hapas*. As vegetable oils are reported to be safer than mineral oils,[2] a series of experiments was carried out with a few selected common vegetable oils to ascertain whether these Notonectids could be effectively controlled in ponds without adversely affecting the other living animals including fish. The results obtained are briefly detailed in this note.

Experiments were carried out in glass jars containing 3 litres of pond water, and having a surface area of approximately 0·2 sq. ft. 5 Insects were introduced in each jar. The results of application of different doses of selected oils are given in Table I.

Linseed and mustard oil gave the most satisfactory results as regards killing of the insects. But under laboratory conditions linseed oil was found to be slightly toxic to the early fry as well as zooplankton. Owing to their

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TABLE I. *Details of Laboratory Experiments Showing the Effects of Vegetable Oils on* Anisops *spp.*

Oil used	Dose per acre of water surface	Reaction on insects		
		Obvious signs of distress	First specimen died in	Complete kill in
	lbs.	Min. after application	Min. after application	Min. after application
Mustard oil ..	50	5	8	23
Cocoanut oil ..	50	6	8	21
Castor oil ..	50	3	7	30
Til oil ..	50	6	10	30
Groundnut oil ..	50	7	10	26
Linseed oil ..	50	5	6	16
Mustard oil ..	25	7	13	29
Coconut oil ..	25	14	30	1 hr. 40
Castor oil ..	25	10	27	1 hr. 19
Til oil ..	25	10	19	1 hr. 37
Groundnut oil ..	25	16	25	1 hr. 40
Linseed oil ..	25	6	8	20

high viscosity the vegetable oils spread very slowly on the water surface. Emulsions of the different oils were therefore prepared by the addition of soap wastes or ordinary cheap washing soap solution, and this facilitated easy spreading of the oil as a thin film over the water surface. A quantity of soap equal to about $\frac{1}{3}$ the weight of oil was sufficient to make a satisfactory emulsion. The results of the field trials in nursery ponds are detailed in Table II below.

For successful results the oil has to cover the entire water surface for some time as a thin film. However, under field conditions, wind is likely to break the surface film of oil, enabling some insects to survive. The application of oil in small nursery ponds, in the absence of a strong wind should therefore give satisfactory results. Though, under laboratory conditions, a very light dose (Notonectids are completely killed in about 55 to 56 min. with mustard or linseed oil @ 12 lb. per acre) is effective, a minimum of 50 lb. per acre may be considered a satisfactory dose under natural condi-

TABLE II. *Details of Field Experiments to Control Notonectids in Nursery Ponds by the Application of Vegetable Oil Emulsions*

No.	Water spread of pond	Oil added	Dose per acre	Quantity of oil added	Quantity of soap mixed	First vissible reaction on insects	Mejority died within	Almost complete kill within
	Acre		lb.	lb. oz.	oz.	Min. after application	Hrs. after applicaion	Hrs. after application
1	0·05	Linseed oil	25	1 5	7	15	4	24
2	0·07	Mustard oil	25	1 11½	9	50	6	26
3	0·055	Linseed oil	25	2 10	14	8	2 15 min.	20
4	0·056	Mustard oil	25	2 12	15	10	3	20

tions in small ponds. Water stick insect (*Ranatra* sp.) is also found to be affected with vegetable oils.

Zooplankton and fish fry in the ponds were not adversely affected by the application of these vegetable oils (excepting linseed oil) in the dose specified above. A judicious application of mustard oil in properly prepared nursery ponds, a few days before the introduction of carp fry should, therefore, result in a substantial reduction in the predatory insect population in the ponds and a corresponding increase in the survival of carp fry was naturally to be expected.

Though the mode of action of the oil on these insects remains to be studied, the relatively low cost involved and the easy field applicability of the experiment seem to warrant extensive field trials in the preparation of carp nurseries for increased production of fish seed.

I am greatly indebted to Mr. K. H. Alikunhi for his valuable suggestions in the course of the work. I am grateful to Dr. S. Guha Sarkar for suggestions on certain chemical aspects and to Dr. S. L. Hora for kindly arranging for the identification of the insects.

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CONTRIBUTIONS TO THE BIONOMICS, ANATOMY, REPRODUCTION AND DEVELOPMENT OF THE INDIAN HOUSE-GECKO *HEMIDACTYLUS FLAVIVIRIDIS* RÜPPEL

Part V. The Urinogenital System

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(Received September 16, 1952)

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1. INTRODUCTION

As the Indian house-gecko, *Hemidactylus flaviviridis* Rüppel, is a suitable type of the Reptilia for class study in Zoology in Indian universities, it is necessary to have an accurate, detailed knowledge of its habits, anatomy and development. The author, in a series of papers extending over several years, has already dealt with its systematic position, bionomics and development (Mahendra, 1936 *a* and 1936 *b*), sexual dimorphism (Mahendra, 1935 *c*), mode of locomotion (Mahendra, 1941), endoskeleton (Mahendra, 1935 *a*, 1935 *b*, 1949 and 1950), respiratory and vocal organs (Mahendra, 1947) and

the circulatory system (Mahendra, 1942). In the present paper, he describes the femoral organs, postanal sacs, hemipenes and the urinogenital system in detail.

2. HISTORICAL RÉSUMÉ

While many more or less valuable observations on the various aspects of the urinogenital system of lizards may be found scattered about in the works of old authors, our present-day knowledge of its anatomy and development specially dates back to Leydig (1872), Heidenhain (1874), Wiedersheim (1876) and Braun (1877). Howes (1887) dealt with the vestigial structures of this system in *Lacerta viridis*; Schoof (1888) made a valuable contribution on the urinogenital system of lizards; and Hoffmann studied its development in reptiles (1889) and gave an excellent consolidated account of it in his monumental work (1889) based on the previous authors. The lizards particularly investigated were *Lacerta agilis*, *L. vivipara*, *L. viridis*, *Phyllodactylus europæus*, *Platydactylus facetanus*, *Varanus bivittatus*, *Calotes jubata*, *Amphisbæna fuliginosa*, *Iguana tuberculata*, *Lophura amboinensis* and *Anguis fragilis*.

In later years, Frederici (1897) described the urinogenital system of *Gongylus ocellatus*; Coe and Kunkel (1904), of the limbless lizard; Morgera (1904), of *Lacerta*; Brooks (1906), of certain North American Lizards; Hädge (1917), of *Uroplatus fimbriatus*; Forbes (1941), of the adult male *Sceloporus*, besides the action of implanted pellets of testo-sterone and esterone; and Mulack (1946), of an oviparous and two ovoviviparous species of the genus *Sceloporus*. Hill (1894) recorded the presence of vestigial Müllerian ducts in a full grown male individual of *Amphibolurus muricatus*; Tellyesniczkys (1897), Frankenberger (1923) and Pesce (1935) studied the histological structure of the testis; and Helt (1924), that of the corpus luteum of *Lacerta agilis*. Möller (1909) pointed out the relation between the urinogenital systems of the tortoise *Emys lutaria* and the lizard *Lacerta agilis*, and Zarnik (1910) compared the reptilian kidney with that of *Echidna*. Fleck (1910) studied the development of the urinogenital system in the gecko, *Platydactylus annularius*; Giersberg (1922), the physiology and histology of the oviducts in reptiles and birds, as well as the development of fibres in it; Dornisco (1925), the existence of a sexual segment in the uriniferous tube of the kidney of *Lacerta impuberes*; Hrabowski (1926), the yolk-organs of lizards; Crowell (1932), the ciliation of the oviducts in reptiles; Inouye (1931), the lobulation of the reptilian kidney from a phylogenetic point of view; Herlant (1931 and 1933), the cyclic variations of the testes and the secondary sexual characters in reptiles; and Alverdes (1936), the vas

deferens in lizards. Dutta (1934) has reported a case of unilateral atrophy of testis in *Hemidactylus flaviviridis*; Jacobi (1936), the egg and oviducts of three species of *Lacerta* in connection with an investigation on ovoviviparity in German lizards; and Vialli and Giovanni (1939), the presence of enterochromatic cells in the urinogenital system of *Lacerta muralis*. Van den Broek (1932 and 1938) has given a good résumé of our knowledge of the urinogenital system in vertebrates.

For the anatomy of the cloaca and hemipenes of lizards, the classical papers on the subject by Gadow (1887), Cope (1896) and Unterhössel (1902) still form the best introduction, but an intensive study of these structures in many representative species is necessary. The only recent contributions worth mention are Wöpke's (1931) and Regamcy's (1934) on *Lacerta agilis*, Van der Merve (1944) on *Acontias meleagris*, and McCann's (1946), besides other reptiles, on three species of *Hemidactylus*, *Calotes versicolor*, *Chamæleon zeylanicus* and *Mabuya carinata*.

The glandular structure of the *femoral organs* has been known for a long time. Wagler (1830), Meissner (1832) and Johannes Müller, for example, noted it in the early part of the last century. Leydig (1872), Schæfer (1902), Cohn (1904), Tölg (1905), Félizet (1911) and Ambrus (1930) investigated their histological structure. Duméril (1834) and Boulenger (1890) employed them for taxonomic distinctions. Otth (1833) and Weidersheim (1909) thought that they played a part in clasping. Félizet (1911) and Kampen (1919) emphasized their resemblance to the sebaceous gland of mammals; and Maurer (1895) and Ambrus (1930), to the musk glands of crocodiles.

The occurrence of post-anal sacs and bones is a peculiarity confined only to the family Gekkonidæ. Schlegel (1838), who was perhaps the first to observe them, described them in *Gekko japonicus*. Wiedersheim (1876) discovered them in *Phyllodactylus europæus*. Boulenger (1885) mentioned their presence in *Tarentola* and *Phelsuma*. Noble (1921) gave a short account of them in *Sphærodactylus*. Deraniyagala (1932) recorded them in *Hemidactylus brooki* Gray. Smith (1933) studied them in a number of Oriental Geckoes, and Wellborn (1933) and Brongersma (1933) made a series of extensive, comparative observations on them.

3. Material and Technique

Specimens of *Hemidactylus flaviviridis* for the present investigation were collected at Agra and Pilani at different times of the year. Besides dissections under a binocular microscope, serial sections were cut, 8 microns thick, of

the femoral pores, kidneys, testes with the epididymes, ovaries and the cloaca. These were stained either with eosin and hæmatoxylin or with Mallory's Triple Stain.

4. GENERAL

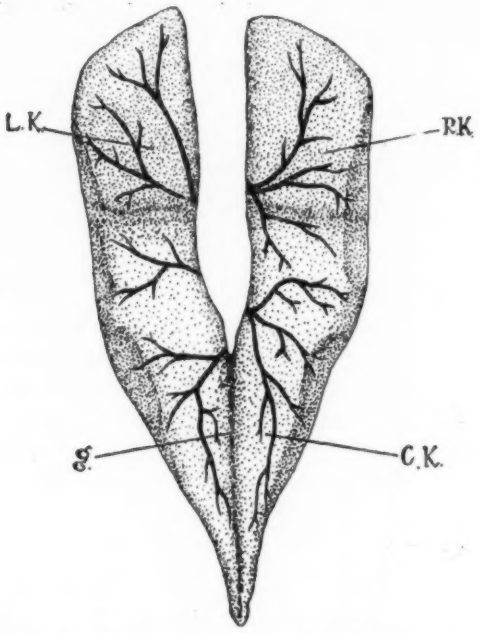
The urinogenital organs consist both of the kidneys and gonads together with their ducts, as well as such accessory structures as the hemipenes, post-anal sacs, femoral glands, etc. Both these systems in vertebrates are generally dealt with together, since they are closely connected to each other morphologically and developmentally.

As is well known, the urinary organs develop in vertebrates as three sets of nephric tubules, one after and behind the other: the *pronephros*, the *mesonephros* and the *metanephros*. In the Amniota, the pronephros is almost always vestigial even in its embryonic condition, but its longitudinal duct (the *pronephric duct*) persists as the mesonephric duct. The mesonephros is functional in the embryo, but it atrophies in the adult. Its duct gives rise to the *Wolffian duct*, which functions as the epididymis and vas deferens in the male, but degenerates in the female. The oviducts (*Müllerian ducts*) arise independently. The metanephros is the functional kidney.

The gonads develop, with no trace of metamerism, from a pair of *genital ridges*, which lie between the mesentery and the nephric fold. In the male, they get connected by a system of channels (*vasa efferentia*) with the kidney tubules, while in the female no such connection is developed.

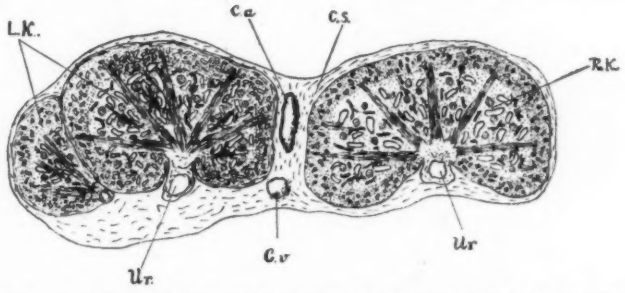
5. THE KIDNEYS AND THEIR DUCTS

The kidneys in *Hemidactylus flaviviridis* are two flattened, elongated bodies of dark reddish-brown colour in the posteriormost part of the pelvic cavity between the dorsal body-wall and the rectum. Their tapering hind ends extend into the tail a little distance posterior to the vent and they are covered ventrally by the peritoneum. When seen from the dorsal side (Text-Fig. 1), they appear roughly triangular in outline, the bases of the triangles being situated mesially. The outer border of each kidney, about half-way back from its anterior end, converges towards the middle line so as to meet the inner border. The external borders curl ventralwards on each side of the rectum, and are divided by a split into an anterior and a posterior lobe. The two kidneys (Text-Figs. 1 and 2) are united intimately together in their posterior halves—a fact not recorded so far. In size, each kidney in the adult measures 10–12 mm. in length and 3–4 mm. at its greatest breadth.



TEXT-FIG. 1. The kidneys of *H. flaviviridis* (Dorsal view). (×6)

C.K., conjoined parts of the kidney, situated mainly behind the anus in the base of the tail; *g.*, groove separating the conjoined kidney; *L.K.*, left kidney; *R.K.*, right kidney.



TEXT-FIG. 2. Transverse section of the kidneys in the region of their posterior, conjoined parts (×60).

C.a., caudal artery; *C.s.*, connective-tissue sheath enclosing and joining the two kidneys; *C.v.*, caudal vein; *L.K.*, left kidney, *R.K.*, right kidney; *U.r.*, ureter.

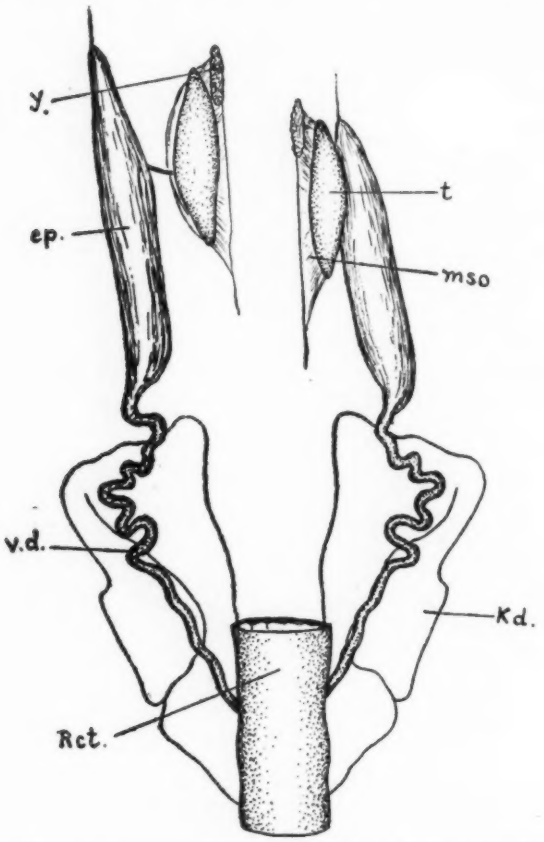
Each kidney has a delicate duct, running on its ventral aspect and opening into the dorsal wall of the cloaca. This duct is the *ureter*. In the male *Hemidactylus* the ureter unites posteriorly with the vas

deferens of its side, the two together opening by a common aperture on a papilla in the urodæal part of the cloaca.

The *urinary bladder* is an extremely thin-walled sac attached by its neck to the ventral wall of the cloaca. Its distal extremity (fundus) is rounded and is attached by a transparent membrane to the ventral wall of the pelvic cavity. The opening of the bladder is separate from the apertures of the ureters.

6. THE MALE REPRODUCTIVE ORGANS

(*a*) *The Testes.*—The testes (Text-Fig. 3) are two white, ovoidal, elongated bodies, lying on each side of the rectum and attached to the dorsal



TEXT-FIG. 3. The urinogenital system of the male *H. flaviviridis* (×3).

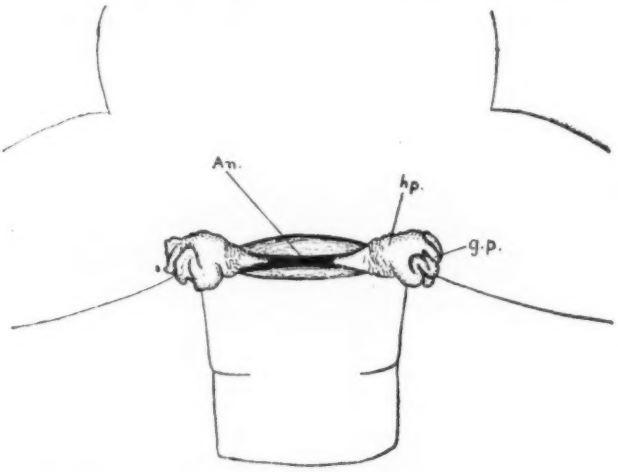
ep., epididymis; *Kd.*, kidney; *mso.*, mesorchium; *Rct.*, rectum; *t*, testis; *v.d.*, vas deferens; *y.*, "yellow body".

body-wall by a fold of the peritoneum, the *mesorchium*. They enlarge in size during the breeding season. The right testis is generally a little larger than the left, and is placed slightly ahead of it.

(*b*) ***The Yellow Body.***—In close association with the testis of each side and situated dorsal to its anterior part, there is a small yellowish-white elongated body at the place where the mesorchium is attached to the dorsal body-wall. This "yellow body" corresponds to the adrenal.

(*c*) ***The Vasa Deferentia and V. Efferentia.***—The *vas deferens* lies laterally on each side, suspended by a fold of the peritoneum. It is much convoluted, and passes over anteriorly into the *epididymis* which, although apparently an elongated, compact body, consists of numerous convoluted tubules. A delicate duct, the *vas efferens*, runs in the peritoneal fold and connects the testis with the epididymis. The vas deferens is comparatively less coiled up posteriorly, runs ventral to the kidney, converges towards its fellow of the other side behind that organ, and opens, along with the ureter, on the apex of a small papilla on the internal side of the dorsal wall of the cloaca.

The structure of the hemipenes has already been described by McCann (1946), and my observations agree with his on the whole, although it must be pointed out that there is a great deal of variation in the appearance of the everted organ and that many of the details mentioned by him are dependent on the degree of the eversion. The pedicil (Text-Fig. 4) is not smooth, as

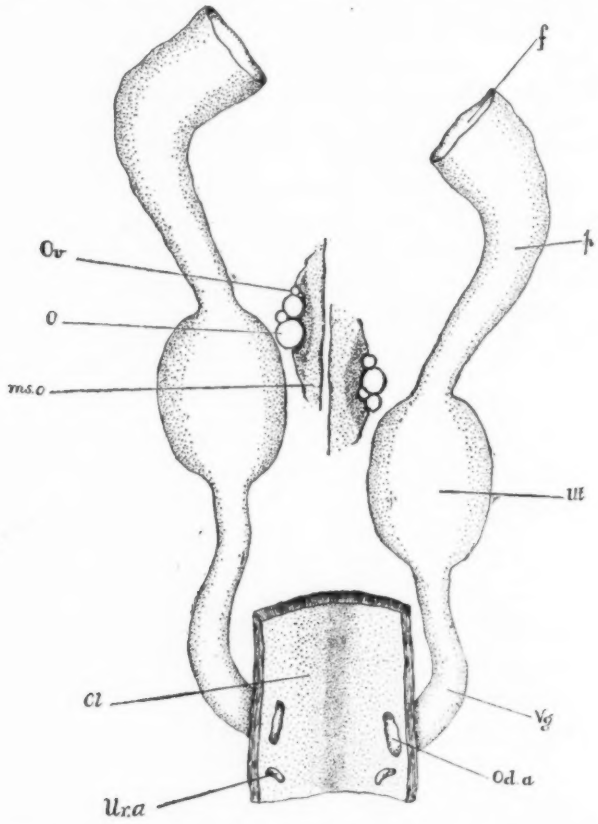


TEXT-FIG. 4. **The hemipenes, everted (×4).**
An., anus; *gp.*, glans penis; *hp.*, pedicil of the hemipenis.

he mentions, but bears a more or less wrinkled appearance a little proximal to the head. There is no transverse ridge, as described by him, on the side opposite to that of the sulcus when the organ is fully everted, and the pattern of ridges on the head is rather variable.

7. THE FEMALE REPRODUCTIVE ORGANS

(*a*) *The Ovaries.*—The *ovaries* (Text-Fig. 5) are situated, one on each side of the middle line, farther back than are the testes in the male, and are both placed almost at the same level. During winter they become inconspicuous, but when the breeding season arrives, they enlarge and become



TEXT-FIG. 5. The reproductive organs of the female *H. flaviviridis* (×3).

Cl., cloaca; *f*, oviducal funnel; *ms.o.*, mesoarium; *O*, developing ova; *Od.a*, aperture of the oviduct in the cloaca; *Ov.*, ovary; *p*, anterior tubular part of the oviduct; *Ur.a*, aperture of the ureter; *Ut.*, uterus; *Vg*, vagina.

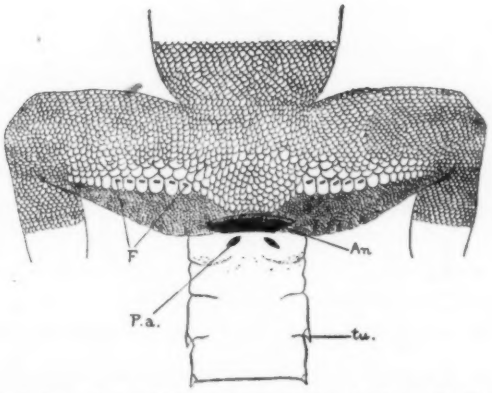
very prominent. Each ovary is an irregularly shaped body with its surface raised up into rounded elevations, and attached to the dorsal body-wall by a fold of the peritoneum, the mesoarium. Usually one, rarely more than one, of the ova contained in it get especially enlarged and develop fully during the breeding season.

Just close to the place where the mesoarium is attached to the dorsal body-wall, there is an elongated yellowish body near each ovary. This is a *vestigial mesonephros* together with the remains of the anterior part of the *wolffian duct*; it consists of a mass of convoluted tubules.

(*b*) *The Oviducts.*—The oviducts lie lateral to the ovaries and are suspended by a fold of peritoneum called the *broad ligament*. Each oviduct can be divided into four parts: the *funnel*, the *anterior tubular part*, the *uterus* and the *vagina*. The *funnel* is the anteriormost part of the oviduct, being an extremely thin-walled division opening into the cœlom obliquely. It leads into the thin-walled *anterior portion* of the duct, which is arranged in the form of flat plaits. The *uterus* or the middle part is a short wide chamber with thicker walls than the rest of the oviduct and has marked longitudinal folds inside to allow for the expansion of this region in order to accommodate the mature egg, which comes to lie and develop here. It is in this part that the earlier development of the embryo inside the egg takes place. The last portion or the *vagina* leads by an aperture into the cloaca.

8. The Femoral Pores

The male *Hemidactylus flaviviridis* has five to seven femoral pores on each thigh along the postero-ventral border (Text-Fig. 6). They are absent



Text-Fig. 6. Scalation of the thighs and anal region in the male *H. flaviviridis* (×2). *An*, anus; *F*, femoral pores; *P.a.*, post-anal sacs; *tu.*, caudal tubercles.

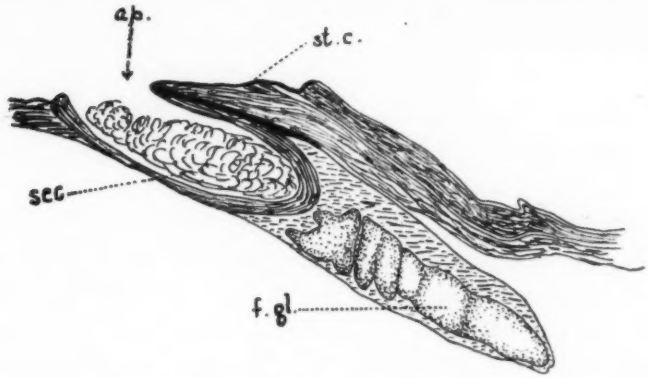
in the female, although the corresponding row of scales in them also is distinguished by an enlargement in size. Posterior to the row of scales bearing the femoral pores (or, in the female, the row corresponding to it), the scales are extremely minute, being subequal and irregular. Smith points out that the femoral pores "have been met with occasionally in what appear to be otherwise normal females". I have not, however, so far come across a single case of female *Hemidactylus* bearing these pores, although I have examined several hundred specimens.

The number and arrangement of the pore-bearing scales varies considerably in different species of *Hemidactylus*. Some (*e.g.*, *H. turcicus*, *H. persicus*, *H. gracilis*, *H. reticulatus*) have pores on a preanal row of scales (*preanal pores*). Others (*e.g.*, *H. flaviviridis*, *H. leschenaulti*, *H. depressus*, *H. giganteus*, *H. bowringii*, *H. karenorum*) bear them on the ventro-posterior aspect of the thighs (*femoral pores*, *sensu stricto*). Still others (*e.g.*, *H. triedrus*, *H. subtriedrus*, *H. brooki*, *H. prashadi*, *H. frenatus*) have a mesially continuous or discontinuous series of what are known as *preano-femoral pores*. The number of these pores may vary from 2 in some rare specimens of *H. turcicus* to 44 in some individuals of *H. giganteus*.

Smith (1936) believes that the pores have little taxonomic value, although —one may point out—that they are often utilized as distinctive features by many taxonomists. Duméril (1834) mentioned that the presence and absence of these apertures serve to distinguish genera and that their number distinguishes species. Boulenger (1890) used these features in many of his synopses for Saurian families including the family Gekkonidæ, while Smith himself (1935) has, to some extent, relied upon them.

In *Hemidactylus flaviviridis*, each femoral pore (Text-Fig. 7) is more or less ovoidal in outline and leads into a cylindrical duct, the *femoral duct*, which is directed obliquely towards the preaxial side of the thigh with a marked inclination towards the proximal end. The femoral duct terminates in a mushroom-shaped gland, divided into a number of plates by a series of in-growing connective-tissue septa. The whole organ roughly resembles an umbrella, the duct corresponding to its handle and the gland to its disc. When the glandular activity is in full swing, there appears inside the interior of the duct a rod-like mass of cellular secretion which may even project slightly out of the femoral pore. The secretion is particularly abundant during the breeding season and appears to be horny in nature.

The function of the femoral organs is not yet definitely known. Some believe that they represent a vestigial organ of no importance in the life of the lizard (Maurer, 1895; Tölg, 1905), while several others concede a



TEXT-FIG. 7. Transverse section through a femoral pore (×35). *ap.*, aperture of the femoral gland; *f. gl.*, femoral gland; *sec.*, secretion of the femoral gland; *st.c.*, stratum corneum.

functional significance to them. Leydig (1872) compares the secretion plug inside them to the pearl organ of certain fishes and to the hairs of mammals. Otth (1833) thinks that they resemble the horny swellings on the fore-feet of frogs which play an important part in mating. Wiedersheim (1909) regards them as clasping organs. Maurer (1895) and Abraham Ambrus (1930) hold that they are a sort of musk-organs, enabling the sexes to locate each other.

It is a fact of some phylogenetic importance that the femoral organs of lizards show a histological similarity to the hairs of mammals. Leydig (1872), in opposition to Maurer's view that mammalian hairs are derived from epidermal sense-organs, regards the femoral organs as a transitional form between ordinary epidermal proliferations and hairs. This is strongly supported by Kampen (1919) who stresses the similarity of these organs to sebaceous glands and is inclined to believe that both hairs and femoral glands have had a common origin.

9. THE POST-ANAL SACS

The post-anal sacs (Text-Fig. 6) are a pair of minute pouch-like organs, situated and opening posterior to the vent. In the female *Hemidactylus*, they are not only much smaller but open by minute slit-like apertures well-nigh within the posterior lip of the proctodæum. In the male they are quite prominent and have their outer rims more or less protruded owing to the presence of a curved bone inside (Text-Fig. 8).

Curiously enough, the position and structure of the post-anal sacs appears to correspond with the anal glands of *Sphenodon*. It would be too

TEXT-FIG. 8. Bones of the post-anal sacs (drawn from an alizarin-stained preparation) (× 8)

much to say in the present state of our knowledge that the two are homologous, but in case they are, Osawa's opinion that the anal glands of *Sphenodon* and the musk-glands of Crocodiles should be homologised with the Saurian penis, must be regarded as untenable, since both penes and sacs co-exist in the family Gekkonidæ.

The function of these organs is problematic. Smith (1933) discovered the presence of glandular cells in them and thought that they produced secretion at the breeding season. Wellborn (1934) regards them as reservoirs for storing sperms or secretion. Noble and Bradley (1933) are of opinion that they should be regarded as stimulating organs which tend to quieten the female during the act of copulation. Wiedersheim (1876), Schmidt (1912) and (to some extent) Brongersma (1934) favour the view that the post-anal bone serves to close the penis-canal during copulation.

10. SUMMARY

1. The two kidneys are intimately united in their posterior halves, and extend a little posterior to the vent. They are divided by a notch into an anterior and a posterior lobe.

2. In the male, each *ureter* unites with the vas deferens of its side before opening into the cloaca. The *urinary bladder* opens separately from the apertures of the ureters.

3. The right testis is usually a little larger. It is situated rather anterior to the left testis.

4. A small " yellow body " is present.

5. The *vasa deferentia*, *epididymes*, *vasa efferentia* and hemipenes have been described.

6. The *ovaries* lie both at the same level farther back than are the testes in the male.

7. A vestigial *mesonephros* is present together with the anterior part of the *wolffian duct*.

8. Each oviduct is divisible into four parts: the funnel, the anterior tubular part, the uterus and the vagina.

9. Each *femoral pore* leads into a femoral duct, terminating in a mushroom-shaped *femoral gland*. The structure of the gland has been described.

10. The similarity of the *post-anal sacs* to the anal glands of *Sphenodon* has been indicated and the various functions so far attributed to these sacs have been mentioned.

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THE ENZYMIC ACTIVITY OF *MACROPHOMINA PHASEOLI*, (Maubl.), Ashby

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The nature and role of pectic enzymes in fungal diseases of plants were dealt with in detail by Brown (1915) and Davidson and Williaman (1927). The present communication relates to the pectolytic activity of *Macrophomina phaseoli* with reference to frenchbean plants and also the loss of coherence observed in cut pieces of potato by enzyme extracts in the fungal mat.

Experimental

Pectin Content of Healthy and Diseased Frenchbean Plants

Earlier studies have revealed the consistent maceration of the tissues of frenchbean plants infected by *M. phaseoli*. Since pectin forms the important constituent of the middle lamella of the plant cell it was considered worthwhile to study the degradation of pectin in diseased frenchbean plants (artificially infected with *M. phaseoli*—a single pycnidiospore culture of the fungus isolated from a naturally infected frenchbean plant). The following procedure was employed for the estimation of the pectin of frenchbean plants:—

The plant material was collected 3 days after wilting and 3 g. samples, containing 7 to 8 plants without leaves and root system, were used for each estimation following the modified method adopted by Nanji and Norman (1928) of the original calcium pectate method of Carre and Haynes. Simultaneously estimations of the pectin content of the correspondingly healthy plants were carried out.

Table I

Percentage pectin content of frenchbean plants, healthy and diseased

	Replicates			Mean
Diseased ..	1·4	1·36	1·09	1·283
Healthy ..	2·41	2·44	2·54	2·466

The results presented in Table I show that the pectin content of the diseased plants is only about 50% that of the healthy ones, and it was therefore decided to study the enzyme degradation of pectin *in vitro* by enzyme extracts of the fungal mat.

The Loss of Coherence in Potato Tissue by M. phaseoli

In the present *in vitro* studies on the enzymic activity of *M. phaseoli* the fungus was grown for 48 hours in frenchbean plant extract (Frenchbean plants 200 g., distilled water 1,000 ml. autoclaved at 15 lb. pressure for 15 minutes).

Enzymic Preparations

(1) *Endo-cellular Enzyme.*—The fungal mat was harvested after 3 days growth, washed in distilled water and filtered over Buchner funnel, pressed between folds of filter-paper and the wet mycelium extracted with phosphate buffer (KH_2PO_4-NaOH) at the following pH levels—pH 5·8, 6·4, 7·0 and 7·6—20 mg. of the wet fungal mat was thoroughly macerated in 20 ml. of buffer and centrifuged at 3000 revolutions for 20 minutes. Both the centrifugate and the mycelial residue suspended in the same buffer were tested for the endo-cellular enzyme activity by Brown's (1915) method, to assess the completeness of the extraction of the enzyme.

(2) *Exo-Cellular Enzyme.*—The culture filtrate after the growth of the fungus was dialyzed and tested in the same way for the presence of the exo-cellular enzyme activity.

The time taken by the different preparations to macerate the substrate (potato tissue) at 37° C. are presented in Table II.

The maceration of the potato tissue observed in the enzyme preparations resembling the condition occurring in the plants during pathogenesis is possibly due to the activity of the fungal enzyme since no such effect was produced either by water or buffer solutions or the inactivated enzyme preparation. The lack of the macerating capacity in the mycelial residue of the dry dehydrated mat (with acetone and ether) indicates that the macerating effect of the mycelial residue of the wet mat was due to the enzymic activity of the fungus remaining alive in the test suspension and not due to incomplete extraction. The exo-cellular enzyme present in the fungal filtrate had poor activity, probably because it was present in low concentrations. pH 6·4 of the buffer was found to be optimum for extracting the enzyme and further experiments were carried out at that pH.

TABLE II

Enzyme preparations	pH	Time in hours		
			Controls	
			Buffer	Water
Wet mycelial extracts in buffer solution	5·8	No maceration after 40 hours	Nil	Nil
	6·4	16	Nil	Nil
	7·0	40	40	Nil
	7·6	40	40	Nil
Wet mycelial residue in buffer solution	5·8	40		
	6·4	40		
	7·0	40		
	7·6	40		
Wet mycelial extract in water	Variable pH recorded between 6·8–7	20		
Wet mycelial residue in water	do	16		
Dialized culture filtrate	do	Poor maceration after 40 hours		
Inactivated extract		Nil		
Dehydrated mycelial extract in buffer solution	6·4	16		
Dehydrated mycelial residue in buffer solution	6·4	Nil		

Observations of repeated trials show that in the higher concentration of the extract the enzymic activity was more pronounced as lesser time was taken to macerate the substrate. The coherenace of the potato tissue was lost in 16 hours, 7 hours and 3 hours in fungal extracts of 20 mg., 250 mg. and 500 mg. of wet mat in 20 ml. of buffer respectively.

DISCUSSION

Experimental results suggest that *M. phaseoli* belongs to the group of fungi whose pathogenesis is related to the production of pectic enzymes which attack the middle lamella and result in the loss of coherence of tissue. It is quite probable that the fungal extract may contain enzymes, other than

pectic enzymes, playing important role in the pathogenesis. Convincing evidence of the existence of three distinct pectic enzymes namely protopectinase, pectase and pectinase was recorded in fungi, *viz.*, *Rhizopus tritici*, *Sclerotium cinerea* and *Botrytis cinerea* (although all three of them were not produced by the same fungus) by Davidson and Williaman (1927). Further work on the enzymic activity of the extracts on pectin and related substances is underway to identify the enzymic constituents of the fungal extracts with these and will be reported later. The experimental results of low pectin content of frenchbean plants infected with *M. phaseoli* (Table I) resulting in complete loss of coherence suggest that the fungus definitely produces pectolytic enzymes during plant infection.

SUMMARY

1. The pectin content of frenchbean plants infected with *M. phaseoli* was 50% less than that of correspondingly healthy plants.

2. That the loss of pectin in the diseased frenchbean plants was due to pectolytic enzyme activity of the pathogen was proved by *in vitro* studies on cut bits of potato with enzymic extracts of the fungus.

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SOIL CONDITIONS AND ROOT DISEASES

IX. Fungal Ecology of Cultivated Fields—Techniques*

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DESPITE the fact that a selective action of surface-sterilising agents on regeneration on the agar plate of root-infecting fungi was indicated by the investigations of Mead (1933) and Davies (1935), much use of that knowledge has not been made by soil mycologists. Sadasivan (1939) used $HgCl_2$ as the surface-sterilising agent for studying fungal succession in decomposing wheat straw and postulated a possible seasonal variation in the relative colonising activity of *Fusarium culmorum* and other soil fungi. Walker (1941), in a comparative study of the action of surface-sterlising agents like $HgCl_2$, $AgNO_3$ and $CaOCl_2$, failed to confirm this seasonal fluctuation and attributed the variation observed by Sadasivan (1939) to the specifically toxic effect of $HgCl_2$ on *Fusarium culmorum*. Walker further indicated that fungal succession on organic matter was governed by the period of incubation of the plant material in the soil, the surface-sterilising agent used and the duration of treatment. $CaOCl_2$ was found in his experiment to secure the most even distribution of fungal colonies. *Fusarium culmorum* was the dominant coloniser when $CaOCl_2$ was used while it showed low resistance to the action of the more severe surface-sterilising agents like $HgCl_2$ and $AgNO_3$.

Tattersfield (1927) reported that the introduction of one nitro-group into phenol increased to some extent its toxicity to fungi. The *para*-substitution was found more potent than the *ortho*-. Stoddard (1942) succeeded in inactivating the virus of the X-disease of peach by soaking infected bud sticks in solutions or suspensions of urea, calcium 8-hydroxyquinolinate, *ortho*-nitrophenol and sodium thiosulphate. Horsfall (1945) in his discussion on fungicides and their action has illustrated the toxicity of various organic and inorganic compounds.

The above observations indicated the need for further experimentation on the efficiency of the root-burial technique for studying soil fungi. The

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following experiments were, therefore, planned to study the influence of different surface-sterilising agents and different periods of incubation on fungal colonisation of cotton roots buried in wilt-sick soil.

MATERIALS AND METHODS

Soil used.—Wilt-sick cotton soil from Udamalpet, South India.

Technique employed.—General laboratory technique on the line proposed by Rawlins (1933) and root-burial technique as used by Subramanian (1946). Autoclaved root pieces were used for burial except in special trials where freshly excised root pieces were used after surface sterilisation.

Surface sterilisation.—The general method employed was sterilisation by immersion for 10 minutes in a 1 in 14 aqueous suspension of $CaOCl_2$ (Walker, 1941). The amount of available chlorine was estimated to be 25 mg. per gram of $CaOCl_2$. Various other sterilising agents considered to be weakly fungistatic but strongly bacteriostatic (Horsfall, 1945) were used in experiments I and II. Solutions were made up in acetone or water depending on the solubility of the substance. The concentration and duration of treatment in each case are given with the results in Tables I and II. All treatments were immediately followed by thorough washing in six changes of sterile tap water. A set of root pieces treated with $AgNO_3$ and NaCl (Walker, 1941) was plated unwashed after treatment.

Results were statistically analysed (Paterson, 1939) on the basis of the numbers of fungi developing on a group of 5 root pieces in all experiments while in Experiment I alone, the results were analysed on single root basis as well.

RESULTS

Experiment I.—The influence of surface-sterilising agents on the development of fungi from autoclaved cotton root pieces buried in wilt-sick soil for 6 months was studied. The results are presented in Table I.

The results show a great deal of variation in the number and type of fungi developing on root pieces treated with different surface-sterilising agents. A two-minutes' immersion in 0·1% $HgCl_2$ (aqueous solution) appeared too drastic a treatment to allow the development of many fungi including *Fusarium*. The $AgNO_3$ treatments showed similar adverse effects. $HgCl_2$ (Fred and Waksman, 1928), *para*-nitrophenol (to be termed *p-np* for sake of brevity), $CaOCl_2$ and sodium benzoate allowed the development of a large number of fungi, at the same time eliminating bacteria from the cultures. However, sodium benzoate was bactericidal only in the higher concentration of 0·1% which to a certain extent inhibited the development of fungi as well.

TABLE I

Surface-sterilising agents used and fungi developed from buried cotton roots

	Surface-sterilising agent used	Concentration	Time of immersion	Average No. of fungi on single root piece	Average No. of fungi on group of 5 root pieces	Average No. of fungi on entire group of 20 root pieces	No. of fungi per root piece		Presence of bacteria	Percentage root pieces yielding	
							Maximum	Minimum		*Fusarium* sp.	Other fungi
		%	Min.								
1	$HgCl_2$ (Fred and Waksman) ..	0·1	1	4·8	7·5	9	8	3	−	100	48
2	do (do) ..	0·1	2	3·9	5·7	7	6	3	−	100	49
3	do (Aqueous) ..	0·1	1	3·9	5·3	7	6	2	+	100	49
4	do (do) ..	0·1	2	2·0	3·0	3	3	1	−	40	80
5	$CaOCl_2$ (do) ..	1/14	10	4·3	6·0	8	6	2	−	100	46
6	$AgNO_3$ (do), NaCl ..	1·0	2	3·2	4·5	6	5	2	−	85	27
7	do (do), do , H_2O ..	1·0	2	2·8	5·0	6	5	0	−	85	37
8	Sodium benzoate (aqueous) ..	0·04	2	4·2	5·3	6	6	2	+	100	64
9	do (do) ..	0·1	2	3·5	4·8	7	5	2	−	100	42
10	*Para*-nitrophenol (do) ..	0·04	2	4·4	7·0	10	7	3	−	100	38
11	*Ortho*-nitrophenol (in 4% acetone) ..	0·04	2	3·4	6·3	9	5	2	+	100	36
12	Calcium 8-hydroxyquinolinate (in 4% acetone) ..	0·04	2	3·4	5·0	6	5	2	+	100	48
13	Magnesium 8–hydroxiquinolinate (in 4% acetone) ..	0·04	2	3·2	5·0	6	6	2	+	100	43
14	8-hydroxyquinolinate (in 4% acetone) ..	0·04	2	2·6	4·3	6	4	2	+	100	32
15	Acetone ..	4	2	3·6	5·8	8	5	3	+	100	37

C.D. at 5% level = 0.6 (No. of fungi on single root basis): (1), (10), (5), (8), (2), (3), (11), (15), (9), (12), (6), (13), (7), (14), (4)

C.D. at 5% level = 1·4 (No. of fungi on 5 root basis): (1), (10), (11), (5), (15), (2), (8), (3), (12), (13), (7), (9), (6), (14), (4).

Immersions of 1 minute in 0·1% $HgCl_2$ (Fred and Waksman, 1928), 2 minute in 0·04% *p-np* and 10 minutes in 1 in 14 aqueous suspension of $CaOCl_2$ appeared the most effective treatments for the development of the maximum number of fungi (including Fusaria) and for the elimination of bacteria. In the $HgCl_2$ and *p-np* treatments *Fusarium* sp. was overgrown by various other fungi, whereas in the $CaOCl_2$ treatment, *Fusarium* sp. was dominant organism exhibiting a very dense growth. Among other fungi, *Macrophomina phaseoli* (*Rhizoctonia bataticola*), *Mucor* sp., *Aspergillus* spp., *Penicillium* sp. and *Syncephalastrum* sp. were predominant. These observations indicate the superiority of the $CaOCl_2$ treatment in colonisation studies involving the isolation of *Fusarium* spp.

Experiment II.—The depressing effect of $HgCl_2$ on *Fusarium* colonisation was further seen when freshly excised root pieces were treated with $HgCl_2$ or $CaOCl_2$ prior to burial and after 24 hours incubation in the soil. The results are presented in Table II.

TABLE II

Fungi developed on root pieces subjected to different pre-burial and pre-plating treatment (period of incubation in soil 24 hours)

Pre-burial treatment	Pre-plating treatment	*Fusarium* (Average on 5 root basis)	Percentage colonisation of		Percentage root pieces not colonised
			Fusarium	*Macrophomina phaseoli*	
1 $CaOCl_2$	$CaOCl_2$	5	100	95	0
2 $CaOCl_2$	$HgCl_2$	5	100	70	0
3 $HgCl_2$	$CaOCl_2$	2·25	45	45	30
4 $HgCl_2$	$HgCl_2$	2·0	40	40	25

C.D. at 5% level 0.6. (1), (2), (3), (4).

The $CaOCl_2$ pre-burial treatment allowed the maximum development of the primary colonisers namely *Fusarium vasinfectum* and *Macrophomina phaseoli*. The pre-plating treatments did not show any significant difference in the fungi developed on the root pieces.

Experiment III.—In an attempt to determine the effect of pre-burial treatments of plant material on fungal colonisation, root pieces were autoclaved or surface-sterilised before burial. In this experiment all surface sterilisations (both before and after incubation in the soil) were effected

treating with 1 in 14 aqueous suspensions of $CaOCl_2$. The results are presented in Table III.

TABLE III

Fungal colonisation of autoclaved and surface-sterilised root pieces after different periods of incubation in soil

	Treatment	Period of incubation in days						
		1	2	3	4	5	6	7
Average No. of fungi on a group of 5 root pieces	A*	2·4	2·3	3·1	2·7	2·6	2·8	2·6
	S. S.‡	1·4	1·7	1·2	1·7	1·7	1·7	1·8
Average No. of fungi on entire group of 20 pieces	A.	4·0	4·0	4·0	4·0	4·0	4·0	4·0
	S. S.	2·0	2·0	2·0	2·0	3·0	2·0	3·0
Percentage colonisation by *Fusarium vasinfectum*	A.	100	100	100	100	100	100	100
	S. S.	100	100	100	100	100	100	100
Percentage colonisation by *Macrophomina phaseoli*	A.	100	100	100	80	86	74	50
	S. S.	40	36	4·0	46	42	26	18
Percentage colonisation by Other fungi	A.	6·0	6·0	17	7·0	7·0	12	18
	S. S.	..	..	..	..	4·0	..	2·0

*A .. Autoclaved.

‡S.S. = Surface sterilised.

It is evident from the results that *Fusarium* (*F. vasinfectum*) is the dominant coloner irrespective of the pre-burial treatment given. However, the autoclaved root pieces are easily colonised by *Macrophomina phaseoli* (100% colonisation in 24 hours) and other fungi like *Mucor* sp. and *Aspergillus niger*, while colonisation of the fresh excised surface sterilised root pieces by these fungi is delayed for a considerable time.

Similar trials in which the incubation period was varied from 1 to 8 weeks also showed a high percentage *Fusarium* colonisation irrespective of the time of incubation of the plant material in the soil while other fungi namely, *Macrophomina phaseoli*, *Mucor* sp., *Aspergillus niger*, *A. fumigatus*, *A. terreus*, *Cunninghamella* sp., *Penicillium* sp., *Trichoderma viride* and *Curvularia lunata* showed a great deal of fluctuation in their colonising capacity.

DISCUSSION

Different methods have been adopted for studying the occurrence of fungi in soils (Waksman, 1922 *a* and *b*, 1927; Conn, 1922). The difference

in behaviour exhibited by soil fungi on natural substrata and on artificial culture media prompted Sadasivan (1939) to introduce his " straw burial " technique which was employed later by Walker (1941) and Subramanian (1946).

The ability of a fungus to colonise buried plant material would be primarily dependent on its ability to decompose cellulose or other materials which are the chief constituents of plant parts. Besides, Walker (1941) has emphasised the importance of surface-sterilising agents and the duration of treatment to which colonised plant materials are subjected in evaluating results on fungal colonisation. He observed that surface sterilisation of buried straw by $HgCl_2$ and $AgNO_3$ proved too severe a treatment and that they inhibited the development of various fungi, especially *Fusarium culmorum*. In the present work, experiments on the effects of various surface-sterilising agents on the development of fungi and the elimination of bacteria from buried stubble have shown agreement with Walker's views. The chemical used as well as the time of immersion in the chemical solution exert considerable influence on the development of fungi (Tables I and II). Thus the development of *Fusarium* sp. is inhibited by 2 minutes immersion of the root pieces in 0·1% aqueous $HgCl_2$ or 1% aqueous $AgNO_3$. Also the beneficial effect of $CaOCl_2$ (1 in 14 aqueous suspension) in promoting the development of *Fusarium* sp. and other fungi was quite evident.

The use of $HgCl_2$ and $CaOCl_2$ for surface sterilisation of root pieces prior to burial in soil has shown that $HgCl_2$ retards fungal colonisation (Table III). It is of particular interest to note that the effect of pre-burial surface sterilisation is much more pronounced than that of the pre-plating treatment.

SUMMARY

1. The number of genera of fungi developing from colonised root pieces depends on the concentration of the surface-sterilising agent used and the duration of the treatment given.

2. For the development of the maximum number of genera of fungi, treatments of 2 minutes immersion each in 0·04% *para*-nitrophenol and 0·1% $HgCl_2$ (Fred and Waksman's formula) and 10 minutes immersion in a 1 in 14 aqueous suspension of $CaOCl_2$ have proved superior to several other chemicals known to be weakly bacteriostatic or bactericidal.

3. *Fusarium* spp. and *Macrophomina phaseoli* (*Rhizoctonia bataticola*) are the primary and dominant colonisers on autoclaved and freshly excised surface-sterilised root pieces buried in Udamalpet soil.

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INVESTIGATIONS INTO THE ORGANIC ACID METABOLISM OF *TAMARINDUS INDICA* LINN.

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Although Benjamin Heyne (1815) and Mayer (1878) were the earliest to observe that leaves of succulent plants like *Bryophyllum* exhibit diurnal fluctuation in acidity, the acid content being highest in the morning and lowest in the evening, it is only since the publication of the monumental work of Bennet-Clark (1933) that the phenomenon has come to be widely known as Crassulacean metabolism. Since this publication a large number of workers investigated the acid metabolism of succulent plants and also of a few non-succulent plants. Notable among these are the studies of Vickery, Pucher and Co-workers [Vickery and Pucher (1940); Allsopp (1937); Bennet-Clark and Woodruff (1937); Thomas (1949); Bonner and Bonner (1948); and Somers (1951)]. The work on acid metabolism has been reviewed by Bennet-Clark (1937), Vickery and Pucher (1940) and Bennet-Clark (1949). In his review of 1933 Bennet-Clark expressed the view that all those non-succulent plants which possess high enough concentrations of organic acids also exhibit a Crassulacean metabolism. It has, however, been found in recent years that the behaviour of some non-succulent plants like rhubarb, *Nicotiana* and *Fagopyrum* is not entirely in conformity with this type of metabolism (Pucher, Wakeman and Vickery, 1938; Vickery and Pucher, 1939; Pucher, Wakeman and Vickery, 1939). Investigations have now been extended to *Tamarindus indica* Linn., a plant with non-succulent leaves and showing accumulations of acids both in the leaves and in the fruits, with a view to study how far the acid metabolism of the plant compares with the Crassulacean type. The present paper deals with the titratable acidity changes with age, diurnal and seasonal fluctuations in titratable acidity, *pH* changes with age and the buffering capacity of the leaf sap of *Tamarindus indica* Linn.

Materials and Methods

A *Tamarindus* tree growing in the Andhra University campus at Waltair provided the material for the investigation. It is a healthy tree growing luxuriantly, flowering and fruiting regularly. Vegetative buds were marked as they came up, by means of date labels. As they begin to open and grow,

the leaves that are found at the tip and that come out first are referred to as Group I, then the next bigger leaves as Group II and so on. The age of the leaves increases from the tip to the base of the twig and the leaves from the youngest to the oldest could be roughly divided into 4 or 5 groups each with its own characteristic T.A.N. value as will be seen later. Random samples are taken of leaves in the various age groups.

There exists a difference of opinion among the various authors regarding the relative usefulness of titratable acidity and total acidity determinations in studies of the organic acid metabolism of green plants. Bennet-Clark (1933) believes that changes in titratable acidity give a fairly correct indication of the changes in total acidity as plants do not contain any bases in sufficiently large quantities to neutralise the acids. Moreover, titratable acidity determinations can be made with a greater degree of accuracy and in a shorter time than estimations of total acidity. On the other hand, Vickery and Pucher (1940) remark that " the day is now past when the results of a simple titration can be calculated in terms of malic acid or citric acid and interpreted in any meaningful way ". Thomas (1947) says " Nevertheless, simple titration may continue to give suggestive information." As recently as 1949 Thomas working on *Bryophyllum* relied entirely on titratable acidity. Somers (1951) working on *Kalanchæ daigremontiana* measured only titratable acidity and *pH*. As such in the present studies observations have been made of titratable acidity and *pH* in the leaves of *Tamarindus*.

For measuring titratable acidity of the leaves, about 1 gm. of the material was accurately weighed and ground in a mortar, then boiled, filtered and the filtrate titrated against 1/50 N Na (OH). Brom Thymol Blue (B.D.H. Parstains label) was used as the indicator. It shows the end point in the *pH* range 6-7·6 and is yellow in acid medium. The end point is indicated by the turning of the yellow colour to blue. The filtrate was free from chlorophyll and no interference was met with during titration.

Bennet-Clark (1933) expressed titratable acidity in terms of mg. equivalent per 100 gm. fresh weight of tissue. He used N/50 Na (OH) for his titrations. Thomas (1949), on the other hand, expressed titratable acidity in terms of the Titratable Acid Number (T.A.N.) which he defined as the amount in c.c. of 1/10 N·Na (OH) required to neutralise an extract from 100 m.g. of tissue. In the present investigation the T.A.N. notation was adopted with slight modifications, namely, instead of 1/10 N, 1/50 N. Na (OH) was used and instead of 100 mg., 1,000 mg. of the leaf tissue were used. The changes were necessitated by the relatively low quantities of acid present in *Tamarindus*,

pH of the sap extracted by crushing the leaves was measured by means of the Beckman *pH* meter (laboratory model G).

I. Titratable Acidity of Leaves

(*a*) *Variation with age.*—The T.A.N. values of leaves of different ages were determined and given in Table I. It is observed that the titratable acidity is very low in the youngest leaves and increases rapidly with age. Acidity falls again in the oldest leaves.

TABLE I

Variation in the T.A.N. with the ageing of the leaves

Position of leaf from tip ..	1	4	6	8	10
T.A.N. ..	19·8	25·5	30·2	24·5	22·8

(*b*) *Diurnal fluctuations in acidity.*—In studies of the titratable acidity of leaves at various hours during the day, the leaves were divided into different age groups so that variation due to age might not mask the diurnal fluctuations and the diurnal fluctuations in the different age groups were studied separately. The T.A.N. values of leaves of the different age groups at various hours during the day have been recorded in Table II. The age groups are numbered from I to IV starting from the youngest leaves at the tip to the older ones towards the base of the twig.

TABLE II

T.A.N. Values of leaves of different age groups at various hours during the day

GROUP I

Time ..	7 a.m.	10 a.m.	1 p.m.	4 p.m.	7 p.m.	10 p.m.	1 a.m.	4 a.m.
T.A.N. ..	17·4	18·3	18·1	17·65	17·5	17·85	17·55	17·55

GROUP II

Time ..	7 a.m.	10 a.m.	1 p.m.	4 p.m.	7 p.m.	10 p.m.	1 a.m.	4 a.m.
T.A.N. ..	24·5	26·2	25·5	25·3	25·2	26·2	25·7	26·4

GROUP III

Time ..	7 a.m.	10 a.m.	1 p.m.	4 p.m.	7 p.m.	10 p.m.	1 a.m.	4 a.m.
T.A.N. ..	28·3	28·3	28·3	27·8	28·6	27·73	27·7	28·2

GROUP IV

Time ..	7 a.m.	10 a.m.	1 p.m.	4 p.m.	7 p.m.	10 p.m.	1 a.m.	4 a.m.
T.A.N. ..	21·5	22·5	21·72	21·2	21·9	22·35	22·0	22·1

These observations were made on a bright sunny day in the month of September. These were repeated on several consecutive days of the same month for 2 years and almost identical values were obtained in the various age groups. It will be seen that there is little diurnal fluctuation, the maximum difference between the lowest and highest values in a day never exceeding 6%. In those plants in which Crassulacean metabolism was recorded the variation in acid content between day and night values was usually high, sometimes reaching as high a value as 1260% (Kraus, 1883).

It was generally observed by previous workers that although there may not be any considerable acidity fluctuation in the youngest leaves, the older leaves definitely showed marked diurnal fluctuation (Bennet-Clark, 1933). In the present study, however, no diurnal fluctuations were observed in any of the age groups. The slight variations on either side of the average characteristic of a particular group might be attributed to chance variations.

(*c*) *Seasonal fluctuations.*—T.A.N. was determined for leaves of Group I in the various months of the year and recorded in Table III.

TABLE III

T.A.N. values of Group I leaves in various months of the year

Month ..	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.
Maximum ..	18·3	18·5	21·3	23·9	28·0	26·1	23·0	20·0	17·0	16·8	17·3	18·0
Minimum ..	17·4	16·0	20·0	24·5	27·1	24·2	21·3	18·6	16·0	16·5	16·9	18·2

The T.A.N. values are highest in the winter months of December, January and February when the lowest temperatures of the year are obtained and when the period is preparatory to flowering. On the other hand, under

temperate conditions Allsopp (1937) working on the rhubarb plant found the greatest accumulation of acids in the green parts of the rhubarb plant during the summer months while only slight increase in the acid content is observed in the rhizome in the winter months. Thomas (1947) explained these results by assuming that acids are probably produced and accumulated in the green shoots in the summer and are later translocated to the rhizome. Bennet-Clark (1930) also observed highest acidity in the summer months in *Sedum præltum* and low values in the winter months. The summer is the best growing period in the temperate conditions and so the greatest accumulation is found in that season there while in this tropical plant *Tamarindus indica* the highest acidity is observed in late winter just before flowering which takes place in April and May.

It is also of interest to note that in *Tamarindus* no diurnal variation in titratable acidity has been observed during the course of a day in any month of the year in any of the age groups.

II. pH of the Leaf Sap

(*a*) *Variation with age.*—The *pH* of the sap of leaves of the various age groups was determined and shown in Table IV. The *pH* is seen to decrease with increasing age. Only in the oldest leaves a slight rise in *pH* is observed. These changes are thus concurrent with corresponding changes in titratable acidity with the ageing of the leaf.

TABLE IV

pH *of leaves of various age groups*

Age group ..	I	II	III	IV	V
pH ..	3·5	3·48	3·3	3·12	3·2

(*b*) *Buffering action.*—Titration curve is drawn for leaves of Group III (Fig. 1). It will be seen that fully mature leaves of Group III have a high buffering capacity.

DISCUSSION

Tamarindus indica with a *pH* of 3·1–3·5 falls in the acid group of plants in Small's (1946) scheme of classification. From the titration curve it will be seen that the buffering capacity of the fully grown leaves is of a high order. Sinclair and Eny (1947) working on the buffer properties of citrus peels found that the juice of the pulp has a greater buffering capacity than the peel. So they concluded that in the presence of organic acids the buffering capacity

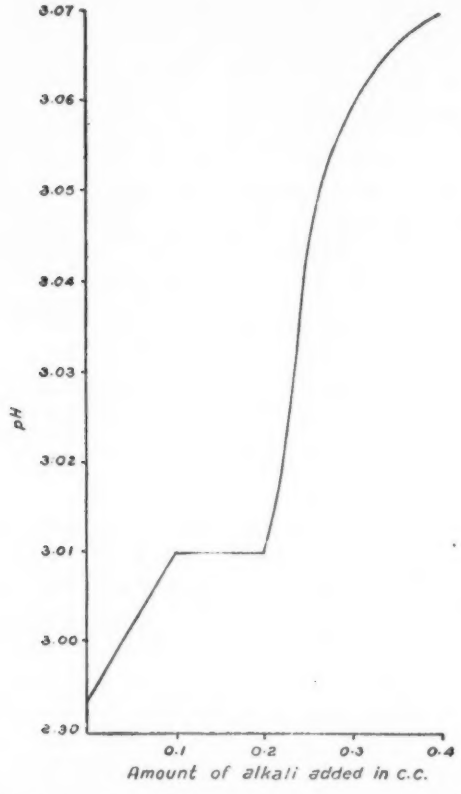

Fig. 1. Relation of change in *pH* to the amount of alkali added in Group III leaves.

is greater than in their absence. The study of the titration curve of the leaf sap of *Tamarindus indica*, therefore, leaves no doubt that in this plant we are dealing with an organic acid metabolism.

The data presented in this investigation show that *Tamarindus* exhibits some of the features of plants having Crassulacean metabolism, namely, (1) increasing titratable acidity and decreasing *pH* with ageing and (2) seasonal variation in acidity. However, there is one important difference—the absence of diurnal fluctuation in acidity. A number of plants have recently been brought to light which do not exhibit diurnal fluctuation in acidity. Thomas (1949) did not observe any diurnal fluctuation in the leaves of *Geranium pratense* which according to Bennet-Clark's list (1933) showed 700% increase in titratable acidity overnight. Bonner and Bonner (1948) failed to observe

the Crassulacean type of metabolism in *Mysembryanthemum edule* and a species of *Sedum* in both of which they found an accumulation of large amounts of acid under all conditions. Bennet-Clark (1933) mentioned that various species of *Mysembryanthemum* were shown to exhibit increase as well as decrease of acidity during night by several authors. *Sedum præltum* was hitherto regarded as a plant showing Crassulacean metabolism. Non-succulent plants like rhubarb, *Nicotiana* and *Fagopyrum* (Pucher, Wakeman and Vickery, 1938; Vickery, Pucher, 1939; and Pucher, Wakeman and Vickery, 1939) were also found to show no Crassulacean metabolism and the real nature of their metabolism is still obscure. Therefore, it seems necessary to devote more attention to this category of plants than has hitherto been done.

Bennet-Clark (1933) suggests that the absence of diurnal fluctuation may be due to either of two reasons; the enzyme system responsible for acid production and depletion is very weak or the rate of depletion of acids is perhaps as fast as that of their formation.

One of the external factors that influences the rate of acid formation and depletion is temperature. Low temperatures generally favour acid accumulation while high temperatures bring about acid depletion (Bennet-Clark, 1933). More recently Somers (1951) came to a similar conclusion. The present study has revealed that *Tamarindus* has greatest titratable acidity in winter months and the lowest in summer months. It is therefore evident that fluctuations in temperature have the same kind of effect on the acid metabolism of *Tamarindus* as of other plants previously recorded. Besides the specific nature of the acid metabolism of the plant it is thus possible that the absence of marked diurnal fluctuations in temperature in Waltair in the different months of the year is also partly responsible for the absence of diurnal fluctuations in acidity.

SUMMARY

The titratable acidity and *pH* of leap sap of *Tamarindus indica* Linn. have been determined under various conditions. The T.A.N.'s of leaves of different ages have been determined and they show an increase with increasing age until in the oldest leaves again there is a slight fall. The T.A.N. values of different age groups have been determined at various hours during the day and it has been found that there is no diurnal fluctuation in acidity in any of the groups. The T.A.N. of leaves of Group I has been determined in the various months of the year. The titratable acidity is highest in the winter months and lowest in summer months. The *pH* shows

a decrease with increasing age until in the oldest leaves again it increases with age. The buffering capacity of the leaf sap is of a high order.

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